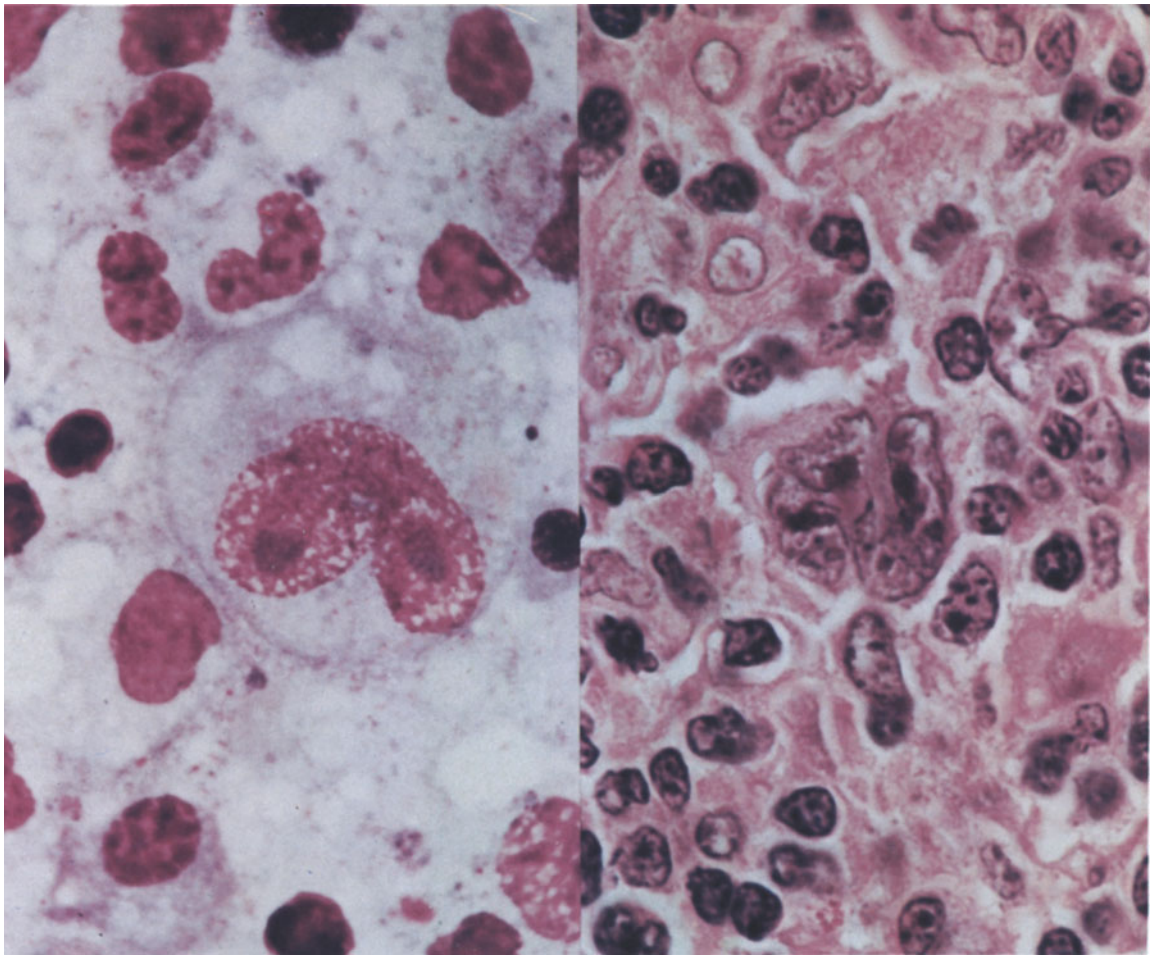

HODGKIN'S DISEASE: Reed-Sternberg cells. Left/Right: Bone marrow aspirate. (Wright-Giemsa, original magnification × 400). Right/Left: Lymph node biopsy. (Hematoxylin and eosin, original magnification × 400). (Courtesy of Powers Peterson).

Diseases of the Lymphatic System

Diagnosis and Therapy

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Preface

This volume presents a practical approach for clinicians who work with patients afflicted with diseases of the lymphoid system and bone marrow. Associated biochemical, hematological, and chromosomal abnormalities, as well as infectious complications, are covered because of their importance in managing patients with lymphatic diseases. In addition, cell kinetics and the classification of lymphocytic lymphomas are included because familiarity with these subjects leads to a better understanding of the physiology of the disease.

Radiological techniques that aid the clinician in the diagnosis and management of diseases of the lymphoid tissue are discussed. The explosive interest in patients with generalized acquired (auto) immune deficiency syndrome (AIDS) prompted the inclusion of a chapter exclusively devoted to this topic. Finally, the chapter dealing with psychological aspects of patients with cancer allows the reader to become familiar with useful techniques.

Distilled from a vast array of data and representing the most up-to-date material available, this volume is intended to be of interest to internists, oncologists, hematologists, radiotherapists, nurses, and anyone in an associated paramedical field who may be involved in the care of patients with diseases of the lymphatic system.

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Plates

Ten examples of the histology of malignant Lymphoma, including Hodgkin's and non-Hodgkin's lymphoma. Sections hematoxylin and eosin stain. (Courtesy of Daniel A. Filippa, M.D.)

L Lukes classification.
WF working formulation classification
Rap. Rappaport classification
Kiel Kiel classification

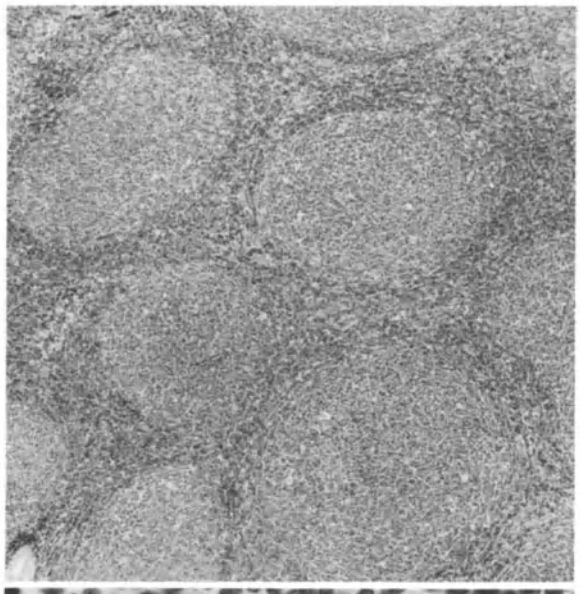
1 poorly differentiated lymphocytic type, nodular (Rap.)
low grade, centroblastic/centrocytic, follicular (Kiel)
FCC, small cleaved cell, follicular (L)
low grade, follicular, small cleaved cell (WF)
(**a**—40×; **b**—430×)

2 well differentiated lymphocytic type, diffuse (Rap.)
low grade, lymphocytic (B-CLL) (Kiel)
small lymphocyte (L)
low grade, small lymphocytic consistent with CLL (WF)
(430×)

3 mixed lymphocytic and histiocytic type, diffuse (Rap.)
low grade, centroblastic/centrocytic, diffuse (Kiel)
FCC, small cleaved cell, diffuse (L)
intermediate grade, diffuse, mixed, small cleaved and large cell (WF)
(430×)

4 histiocytic type, diffuse (Rap.)
low grade, centroblastic/centrocytic, diffuse (Kiel)
FCC, large cleaved cell, diffuse (L)
intermediate grade, diffuse, large cell, cleaved (WF)
(430×)

5 histiocytic type, diffuse (Rap.)
high grade, immunoblastic (Kiel)
FCC, large non-cleaved cell, diffuse (L)
intermediate grade, diffuse, large cell, non-cleaved (WF)
(430×)



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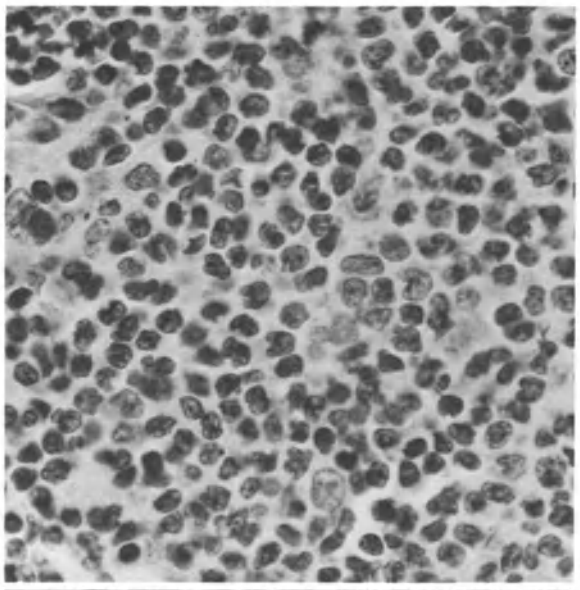

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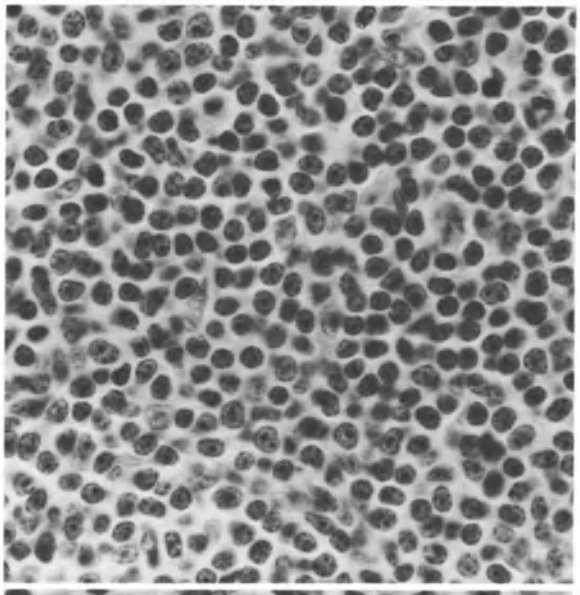

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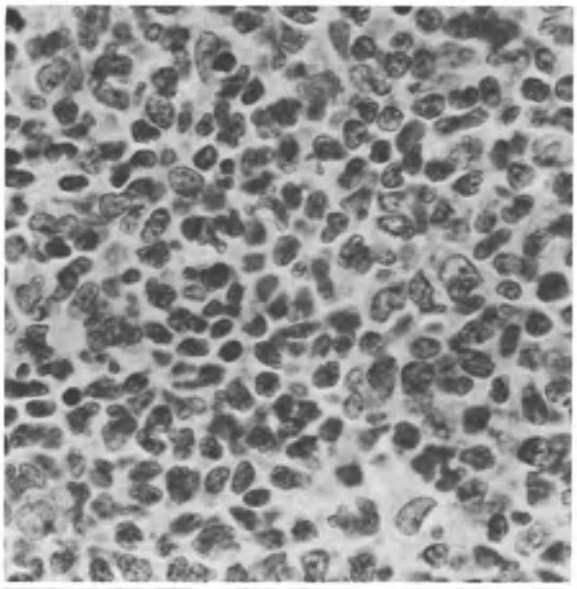

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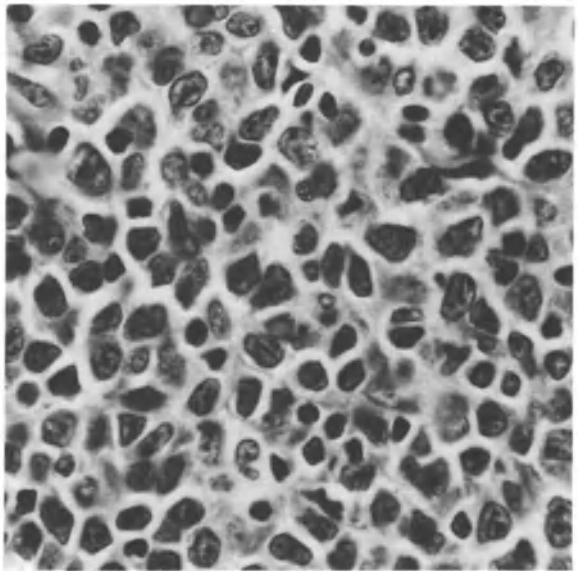

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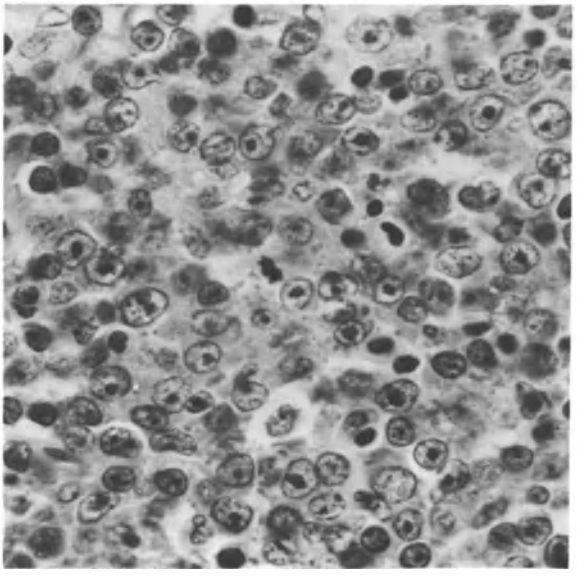

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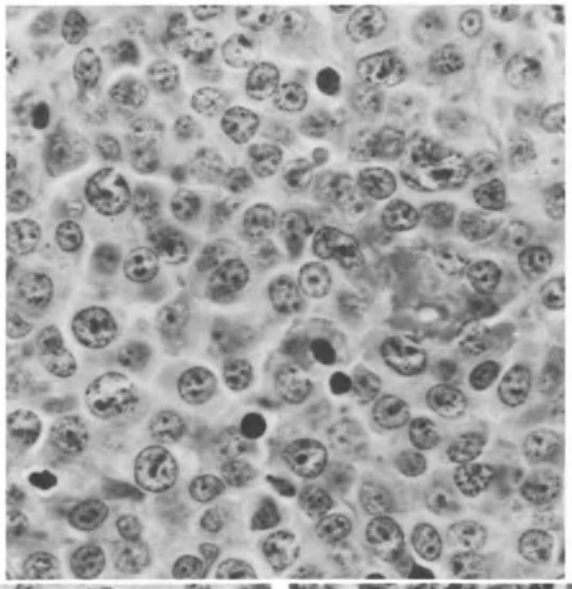
6 histiocytic type, diffuse (Rap.)
high grade, immunoblastic, plasmacytic (Kiel)
immunoblastic sarcoma (L)
high grade, immunoblastic, plasmacytic (WF)
(430×)

7 lymphoblastic, convoluted (Rap.)
high grade, lymphoblastic convoluted (Kiel)
convoluted lymphocytic (L)
high grade lymphoblastic, convoluted cell (WF)
(430×)

8 undifferentiated, diffuse (Rap.)
high grade lymphoblastic, B cell type (Kiel)
FCC, small non-cleaved cell (L)
high grade, diffuse small non-cleaved cell (WF)
(430×)

9 mixed lymphocytic and histiocytic type, diffuse (Rap.)
? T-zone lymphoma (Kiel)
? immunoblastic sarcoma, T type (L)
high-grade immunoblastic, polymorphous (WF)
(430×)

10 Hodgkin's disease (430×)



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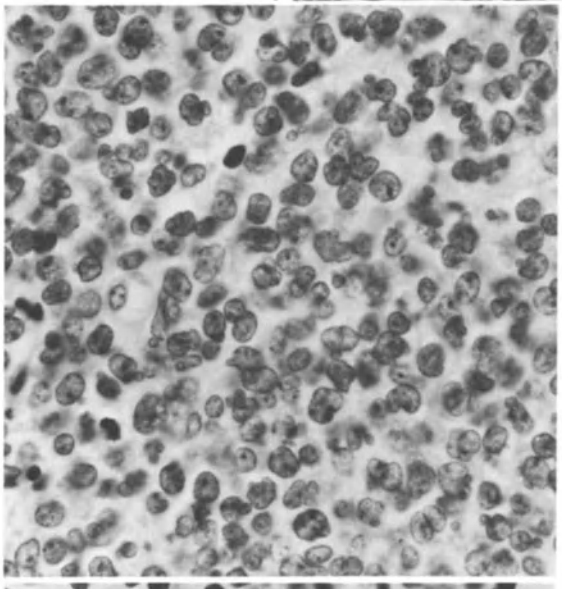

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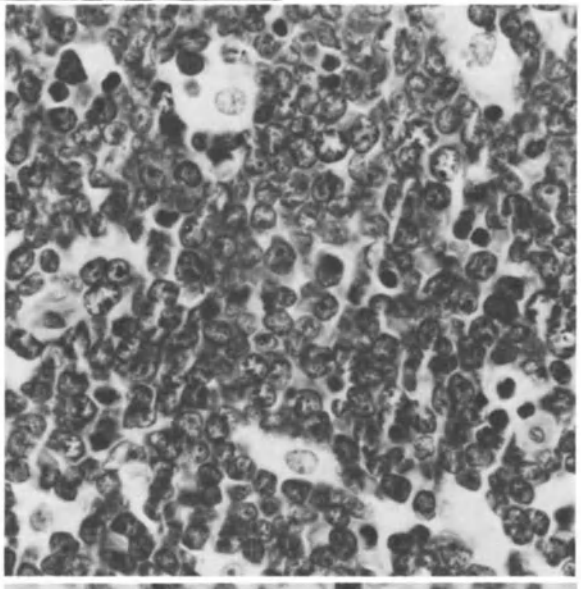

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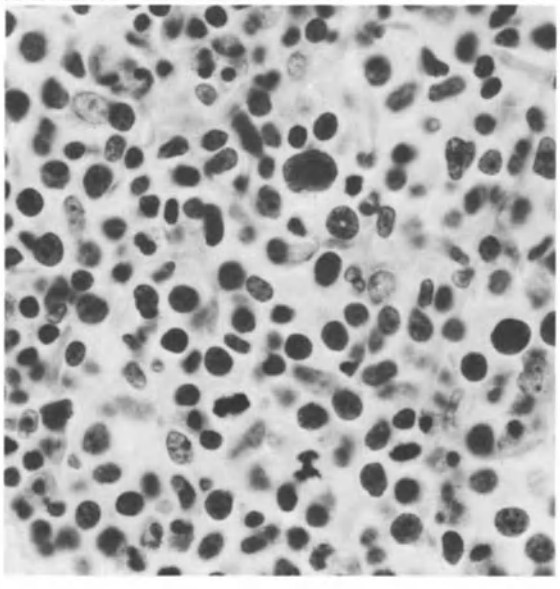

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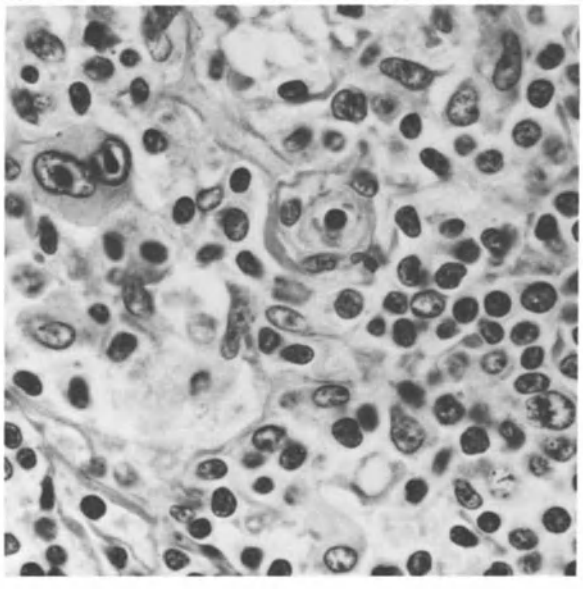

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Abbreviations

AA	amyloid A
ABP	Adriamycin, bleomycin, prednisone
A COMLA	COMLA and adriamycin
AGP	acid α_1-glycoprotein
APRP	acute phase reactant proteins
BACOP	Bleomycin, adriamycin, cyclophosphamide, vincristine, prednisone
BCNU	1,3-bis (2 chloroethyl) 1 nitrosurea
BCOP	BCNU, cyclophosphamide, vincristine, prednisone
β_2M	β_2-microglobulin
CAT	Cytosine arabinoside, adriamycin, 6-thioguanine
CCSG	Children's Cancer Study Group
CHOP	Cyclophosphamide, adriamycin, vincristine, prednisone
CHOP–BLEO	CHOP + bleomycin
C-MOPP COPP	Cyclophosphamide, vincristine, procarbazine, prednisone
CNS	central nervous system
COM	cyclophosphamide, vincristine, methotrexate
CMV	cytomegalovirus
COMA	Cyclophosphamide, vincristine, methotrexate, cytosine
COMLA	arabinoside
COP + Ara C	Cyclophosphamide, vincristine, prednisone, cytosine arabinoside
COP-BLAM	Cyclophosphamide, vincristine, prednisone, bleomycin, adriamycin, matulane
COP CVP	Cyclophosphamide, vincristine, prednisone
CRP	C-reactive protein
CSF	cerebrospinal fluid
CSF	colony-stimulating factor
CT	computerized tomography
DIC	disseminated intravascular coagulation
EORTC	European Organization for Research on the Treatment of Cancer
ESR	erythrocyte sedimentation rate
HCL	hairy cell leukemia
HDL	high-density lipoprotein
HOP	Adriamycin, vincristine, prednisone

LACE	bronchoalveolar lavage fluid levels of angiotensin-converting enzyme
LDH	lactate dehydrogenase
LI	labeling index
L2	Cyclophosphamide, prednisone, vincristine, daunorubicin, cytosine arabinoside, thioguanine, L-asperaginase, BCNU
MBACOD	MBACOP, Dexamethasone replaces prednisone
MBACOP	BACOP and methotrexate
MCV	modal cell volume
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
PWM	pokeweed mitogen
SAA	serum amyloid A
SACE	serum levels of angiotensin-converting enzyme
SCE	sister chromatid exchanges
TACE	tissue levels of angiotensin-converting enzyme
TRIKE	cyclophosphamide, vincristine, methotrexate, cytosine arabinoside

CHAPTER 1

Hodgkin's Disease

BENJAMIN KOZINER

The initial enthusiasm that followed the dramatic progress in the treatment of Hodgkin's disease (HD) has been tempered by concern over the short- and long-term iatrogenic effects. Moreover, this skepticism has been reinforced by the lack of appropriate understanding of the fundamental biologic features and pathogenetic mechanisms of the disease.

Histopathology

Over 150 years ago, Thomas Hodgkin reported on the pathologic findings of seven patients afflicted by the enlargement of lymph nodes and spleen.[75] His name has remained associated with this lymphoid disease since the acknowledgment made by Wilks in his 1865 report.[181] Further definition of the histologic features of the disease and the definitive recognition of the multinucleated giant cell was provided by Sternberg[162] in 1898[162] and Reed[134] in 1902. In 1944, Jackson and Parker proposed the histologic division of HD into three subtypes: paragranuloma, granuloma, and sarcoma.[83] This classification was replaced by the one proposed by Lukes and Butler[109] in 1966, and subsequently by the Rye modification of this scheme, which is currently in use.[110] Basically, four histologic subtypes are recognized: lymphocyte predominance, nodular sclerosis, mixed cellularity, and lymphocyte depletion. This prognostically significant subgrouping reflects decreasing degrees of lymphoid infiltration and the interaction of different mononuclear cell populations with lack of well-defined criteria of invasiveness characterized by the neoplastic processes.[92,133]

Lymphocyte predominance is characterized histologically by an abundance of small lymphocytes, a scarcity of typical Sternberg-Reed (S-R) cells, and a variable proportion of the "lymphocytic–histiocytic" variant of the S-R cell, according to the Lukes and Butler terminology. The common clinical presentation of lymphocyte predominance is that of high cervical lymphadenopathy in a young asymptomatic male. Both subdiaphragmatic and extranodal involvement are most unusual. This is the most prognostically favorable of all four subtypes.

The main histologic features of nodular sclerosis are the presence of bands of collagenous fibrosis subdividing the lymph node into nodules and the "lacunar" cell variant of the S-R cell. Clinically, nodular sclerosis most commonly afflicts young asymptomatic females presenting with mediastinal involvement. Rarely, there is subdiaphragmatic or, more rarely, extranodal spread. It is prognostically favorable category unless there is massive mediastinal and/or hilar lymphadenopathy.

The mixed cellularity subtype is characterized by cellular pleomorphism with a variable admixture of plasma cells, eosinophils, lymphocytes, and histiocytes and a moderate number of S-R cells. A more frequent incidence of subdiaphragmatic and symptomatic "B" disease (see section on Staging) makes the prognosis less favorable than that of the previously described subtypes. However, due to the widespread use of laparotomy and splenectomy with recognition of occult HD, the prognostic differences between mixed cellularity and nodular sclerosis have for practical purposes disappeared.

Both variants of lymphocyte deletion, reticular

and diffuse fibrosis, display a marked depletion of cellular elements, including lymphocytes, with an increase in the reticular framework.[119] Numerous typical and atypical "anaplastic" S-R cells are common, mostly in the reticular variety. Postchemotherapeutic and postradiotherapeutic changes in lymphoid tissue infiltrated with HD tend to resemble both histologic variants of lymphocyte deletion and deserve appropriate distinction. Lymphocyte deletion represents the most aggressive subtype of HD, affecting mainly an older symptomatic population presenting with pancytopenia, anergy, frequent absence of peripheral lymphadenopathy, hepatosplenomegaly, and bone marrow infiltration. Retroperitoneal nodes are often involved.

Although most cases can readily be classified among the four categories described, approximately 15% of the patients present with features that make classification difficult. Among those cases, presumably there were some in which the diagnosis of immunoblastic lymphadenopathy would have been entertained at the present time.

Epidemiology

The common bimodal distribution of HD in the young (two subgroups, with peak risks at 0–14 and 25–30 years, respectively) and the elderly (peak risk over 50 years) is associated with particular histologic subtypes and clinical presentations. This bimodality has been used as an argument to suggest the possibility that these two forms have different etiologic and pathogenetic mechanisms. MacMahon[111] proposed that HD might represent a chronic granulomatous inflammation in young adults and neoplasia in the elderly. According to Gutensohn and Cole,[67] HD in the younger population might present originally as an infection process, probably viral, and acquire malignant potential as the age at infection progresses due to geographic and socioeconomic conditions. The increased incidence of HD observed in relation to reduced family size[68] and tonsillectomy[173] could be explained by these factors.[69]

The possible infective and transmissible potential of HD suggested by the description of "clusters" of HD among high school classmates[172] has been rigorously challenged.[66,159] A high incidence of serum antibodies against the Epstein-Barr virus[73] and herpes virus type II,[25] as well as cytomegalovirus,[98] has been reported in patients with HD. However, these studies have failed to provide convincing evidence that viruses have a role in the etiology of HD, since the viral genome has not been found in involved tissues.[94]

Bjorkholm et al. demonstrated functional T lymphocyte defects in monozygotic and dizygotic twin siblings of HD patients.[10] They also described a similar T cell deficiency in spouses and relatives of patients with HD.[11] Grufferman et al. reported on the higher risk of sex-concordant siblings of patients with HD as compared with the general population.[65] These observations suggest not only a genetic linkage or susceptibility (supported by the increased association with A1, B5, and B18 HLA antigens)[168] but also the horizontal transmission of the causative agent.

Biology

An interesting hypothesis for the pathogenesis of HD was formulated by Order and Hellman almost a decade ago.[124] They proposed that T cells are infected by a tumor-inducing virus that causes a change in cell surface antigenicity. This alteration causes T–T cell interactions, such as occur in the graft-versus-host reaction, that could subsequently lead to lymphocyte depletion and the development of a malignant histocytic line.

Although the neoplastic nature of HD is clinically beyond doubt, its biologic confirmation has been hampered by the failure to identify the predominant malignant cell. Recent evidence based on cytogenetic and cell culture studies suggests that the giant cells in HD exhibit two essential features of neoplastic cells: aneuploidy and clonal origin.

Cytogenetic analysis of lymphoid tissue infiltrated by HD has shown a variable population of aneuploid (often hypotetraploid) cells.[125,135] Moreover, marker chromosomes have also been described.[152] The establishment by Kaplan and Gartner of long-term cell cultures of mono- and multinucleate HD cells derived from involved spleens allowed the detailed analysis of their phenotypic and cytogenetic features.[89] Although the mononuclear HD cells were predominant, multinucleate giant forms resembling S-R cells were also present. Lysozome secretion was consistently observed in all HD cultures tested. The giant cells were adherent and phagocytic, possessed both Fc

and complement receptors (C3b), and lacked lymphocyte markers such as C3d receptors, surface IgM, and the capacity to form sheep erythrocyte (SRBC) rosettes. Although earlier reports argued in favor of the B cell nature of the S-R cell based on the detection of cytoplasmic immunoglobulin, Kadin et al. demonstrated that both kappa and lambda light chains were present intracellularly which were more likely the result of phagocytosis than synthesis.[85] Fibronectin was also detected in the cytoplasm of S-R cells by indirect immunofluorescence with a monospecific antibody.[136] These phenotypic features strongly favor the derivation of S-R and HD cells from the histocyte–macrophage line. Binuclear and multinucleate cells, but mostly the mononuclear HD cells, were capable of active DNA synthesis and binuclear mitotic division.[89] These data differ from previous observations that characterized the S-R cell as a nonproliferating end-stage component of the neoplastic process. Moreover, the giant cells displayed aneuploidy (hyperdiploidy, hypotetraploidy) consistently, and when transplanted into nude athymic mice, resulted in tumor formation with similar phenotypic and cytogenetic features. Although the observations reported by Kaplan's group will require confirmation by other investigators, they provide rational biologic proof of the neoplastic nature of HD.

Schaadt et al. were also able to establish two cell lines with the characteristic morphology of mononuclear and multinucleate S-R cells[147] from pleural effusions of two patients with HD. Both lines complied with the criteria of heterotransplantability in nude mice and a monoclonal pattern of marker chromosomes required for acceptance as putative neoplastic lines. However, in contrast with the observations of Kaplan and Gartner,[89] the only surface markers detected on the cultured cells were "Ia-like" antigen and rosette formation with SRBC-rosetting lymphocytes. Both lines failed to produce lysozyme. These phenotypic features are not compatible with the histiocyte–macrophage lineage proposed by the group at Stanford and await further confirmation.

Immunology

Several studies of delayed cutaneous hypersensitivity in HD have documented an increased but variable incidence of anergy to recall antigens.[18,22,44,184] However, when sensitive skin testing to new antigens was employed, anergy to vigorous antigenic challenge such as 2,4-dinitrochlorobenzene was usually limited to patients with active disease and constitutional symptoms.[1,18,22,184] A significant number of patients with HD are lymphopenic (15%–40%),[2] and this abnormality appears to be more frequently associated with dissemination of the disease and the lymphocyte deletion subtype.[44,184]

Great variability in the number of B and T lymphocyte populations has been reported. However, the lymphopenia seems to be due mainly to a reduction in SRBC-rosetting (T) lymphocytes.[22,39] Fuks et al. reported on the impaired capacity of peripheral blood lymphocytes from untreated patients with HD to form SRBC rosettes and to respond *in vitro* to phytohemagglutinin (PHA) stimulation.[55] Overnight incubation of the peripheral blood lymphocytes in culture media containing 20% fetal calf serum was followed by restoration of the percentage of SRBC-rosetting cells to normal levels and of their capacity to respond normally to PHA. Similar results were observed after incubation in fetal human serum but not in adult human AB serum or adult bovine serum. These findings suggest that there is a specific interaction between serum factors and the surface of peripheral blood SRBC-rosetting lymphocytes in HD.

It had been shown previously that lipoproteins,[32] antilymphocyte antibodies,[64] and C-reactive protein[118] impair SRBC rosette formation. Both C-reactive protein[183] and ferritin[46] levels have been found to be elevated in patients with HD. Furthermore, a similar blocking effect of SRBC rosette formation has been described for ferritin (or apoferritin) that interestingly was reversed *in vitro* by levamisole (an antihelmintic and presumptive immunopotentiator).[117] Bieber et al. at Stanford found that extracts from involved spleens with HD contained a factor inhibitory to SRBC rosette formation.[9] On preliminary analysis it appeared to be a low-density lipoprotein, but upon further biochemical characterization it proved to be a glycolipid.

DeSousa et al. proposed that metal-binding proteins, including ferritin, transferrin, and lactoferrin, have a role in lymphocyte "ecotaxis."[39] This hypothesis attempts to explain the mechanisms governing lymphocyte circulation and tissue distribution on the basis of cellular interactions depen-

dent on surface phenotypes of developmental significance. Iron and ferritin deposits have been detected in tissue sections from spleens involved by HD. Moreover, by immunofluorescent staining, ferritin was also observed in the large binuclear cells of pleural effusions and short-term spleen cell cultures involved by HD.[160] According to DeSousa et al., the main pathogenetic mechanism of the immunodeficiency state in HD might involve the failure of lymph node and splenic monocytes to handle iron and iron-binding proteins normally, resulting in T lymphocyte sequestration.[39] Splenic lymphocytes, in contrast to those from the patients' peripheral blood, show elevated proportions of SRBC rosettes and relatively normal PHA reactivity.[39,112] Splenectomy results in an increase in the peripheral blood SRBC-rosetting cells and a return to normal of the lowered PHA responsiveness observed in untreated patients with HD.[39,175]

The somatic cell hybridization technique for the production of specific monoclonal antibodies[95] has allowed the characterization of functionally distinct T cell subsets. The analysis of peripheral blood lymphocytes in untreated patients with HD was remarkable only for a reduction in the number of total T cells ($T3^+$) in patients with "B" symptoms. Normal proportions of helper ($T4^+$) to suppressor–cytotoxic ($T5^+$) cells were observed and were not altered after splenectomy.[130] By immunoperoxidase staining of tissue sections and indirect immunofluorescence of cell suspensions, Poppema et al. demonstrated that the majority of splenic lymphocytes were of T cell type ($T1^+$, $T3^+$) and most of these bore the helper–inducer phenotypic marker ($T4^+$), with a minority of suppressor–cytotoxic ($T5^+$, $T8^+$) cells.[128] Ia antigen was expressed in the surface of some T cells (suggesting cellular activation) and in the surface membrane and cytoplasm of S-R cells which otherwise failed to stain with the entire panel of T cell monoclonal antibodies.

Impaired lymphocyte function was one of the earliest findings in the immunologic evaluation of HD patients, although it was unclear whether the defect was intrinsic to HD or the result of the immunosuppressive effects of radiotherapy and/or chemotherapy.[37,74] A dose-dependent defect in lymphocyte stimulation by PHA was not only observed in untreated patients with advanced HD, but also in untreated patients with localized disease and in patients free of disease for 2–8 years after radiotherapy for HD.[102] Similar hyporesponsiveness was found with concanavalin A and pokeweed mitogen.[22,44,76] Although the impaired mitogen responsiveness was most consistently reported in patients with advanced stage, it could also be observed with localized disease.[48]

It has been reported that prostaglandin-producing suppressor cells may have a role in the hyporesponsiveness to PHA seen in HD.[62] Addition of indomethacin to PHA cultures of lymphocytes in patients with HD resulted in an increase in thymidine incorporation as compared with controls. PHA cultures of HD lymphocytes produced approximately a fourfold increase in prostaglandin E_2 after 48 hours. Removal of glass-adherent cells markedly decreased the enhancement seen with indomethacin, reduced prostaglandin E_2 production by more than 80%, and eliminated the differences in response to PHA between HD and normal lymphocytes. It was proposed that a glass-adherent, prostaglandin-producing suppressor population is responsible for the PHA hyporesponsiveness seen with HD lymphocytes.

The early literature documented the lack of significant impairment of humoral immunity in HD. Marked abnormalities in the primary antibody response that correlated with clinical prognosis[3,8] were detected only in the advanced stages. Hypogammaglobulinemia is infrequent, and hypergammaglobulinemia is present in approximately one-third of the patients.[115]

Elevated levels of circulating immune complexes were also detected on 50% of untreated patients with HD, but in only 5% of patients in complete remission after treatment.[17] Although no correlation could be established between the level of immune complexes and the presence of constitutional symptoms or histologic subtype, there was a relationship with stage. Elevated levels of circulating immune complexes were observed in stages I–II and III but not in stage IV, presumably due to the failure of antibody production in this advanced stage. Brandeis et al.[13] also described elevated immune complex levels in children with HD, but in contrast to the results reported by Brown et al.,[17] there was progressive increase in levels from stages I to IV.

Longmire et al. reported an increased total splenic IgG synthesis in most patients with HD studied.[106] Mean IgG production by uninvolved or slightly involved spleens was 5 and 11 times normal, respectively, in contrast to only a 2-fold increase over normal levels in heavily involved spleens. When the IgG produced in culture by spleens affected by HD was incubated with homo-

logous lymphocytes, significant IgG binding was found. These data suggest that the spleen in HD responds with antibody synthesis to some antigen associated with lymphocytes. This deranged B lymphocyte reactivity in HD, with the production of antibodies having cross-specificity to nonmalignant (but perhaps virus-infected) T cells, may be partly responsible for the adenopathy found on biopsy to be reactive follicular hyperplasia. Falsely abnormal lymphangiograms and enlarged spleens without evidence of tumor invasion are relatively common occurrences and may be explained by this unusual lymphocyte reactivity. The same group reported that IgG produced in cultures of splenic lymphocytes that were obtained from patients with HD would bind to a population of homologous peripheral blood lymphocytes and initiate antibody-dependent cytotoxicity.[107] This mechanism might explain the anergy and lymphopenia often seen in HD.

A most comprehensive multivariate analysis of T cell function and circulating serum factors in HD was recently reported by Schulof et al.[150] Each of the 31 untreated HD patients studied (15 in stages I–II and 16 in stages III–IV) demonstrated at least one defect in the many parameters analyzed. T cell colony formation, chemotaxis, and PHA responsiveness were the functional studies most frequently found to be abnormal and were generally related to advanced disease. Moreover, more than two-thirds of the patients studied demonstrated elevated immune complex and prostaglandin levels. The latter abnormality was associated with increased PHA responsiveness and depressed T cell colony formation. A significant direct relationship was also observed between serum zinc levels and T cell chemotaxis. Despite the comprehensive investigation undertaken by Schulof et al., their conclusion was that the deranged immunity of HD patients was the result of multiple complex factors, including intrinsic T cell defects.

Although Case et al. reported normal numbers of B and T lymphocytes in HD patients after 5 years in clinical remission following radiotherapy,[23] persistently low numbers of SRBC-rosetting lymphocytes were demonstrated by Fisher et al. in HD patients 10 years after receiving nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP) chemotherapy.[52] However, both radiotherapy and chemotherapy resulted in marked mitogen hyporesponsiveness in the two groups of long-term complete responders. These defects were not observed in patients with histiocytic lymphoma similarly treated and followed, suggesting an intrinsic immunologic defect in HD patients.[52]

Specific antibody response against *Hemophilus influenzae* type B was significantly reduced in patients receiving combined treatment (radiotherapy and chemotherapy), whereas single-mode treatment reduced titers marginally (chemotherapy) or not at all (radiotherapy).[178] Untreated patients had normal values, and splenectomy had no effect. Therefore, aggressive treatment with chemotherapy and irradiation impairs humoral defense against encapsulated microorganisms and thus magnifies the risk of postsplenectomy septicemia in patients with HD. Moreover, the antibody response to pneumococcal vaccine was profoundly impaired in patients who had received intensive treatment for HD.[3] The development of herpes zoster is a frequent event among patients with HD, in the range of 15%–19%, compared to an incidence of less than 0.05% in the general population.[59,182] Its incidence appears to be greater after splenectomy[149] and after completion of radiotherapy,[132] and a small proportion of these patients develop disseminated herpes zoster.

Staging

The original three-stage scheme proposed by Peters[126] to classify prognostically distinct patient groups was replaced by the staging system formulated in 1971 at the Ann Arbor Workshop.[21] This classification was based on observations gathered mostly by the use of lower extremity lymphangiography.[93] This technique enabled the recognition of retroperitoneal node involvement and resulted in an improved understanding of the patterns of lymphatic spread.[99]

The Ann Arbor classification consists of four stages that can be established clinically or pathologically, in the latter instance by histologic confirmation of involvement by HD in multiple biopsies at laparotomy. It should be emphasized that the Ann Arbor clinical staging classification (Table 1–1) applies only to patients at the time of disease presentation and prior to definitive therapy. Both liver and bone marrow involvement are always considered to indicate stage IV. Involvement of other extranodal sites such as bone ($_o$), lung ($_l$), pleura ($_p$) or skin ($_s$) could be considered either an "E" lesion (designated stage number) if localized and contiguous to an involved nodal area,

TABLE 1–1 Clinical Staging Classification

Stage	Definition
I	Involvement of a single lymph node region (I) or of a single extralymphatic organ or site (I_E)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site and one or more lymph node regions on the same side of the diaphragm (II_E)
III	Involvement of lymph node regions on both sides of the diaphragm (III), which may also be accompanied by involvement of the spleen (III_S) or by localized involvement of an extralymphatic organ or site (III_E), or both (III_{SE})
IV	Diffuse or disseminated involvement of one or more extralymphatic organs or tissues, with or without associated lymph node involvement

or stage IV disease if multiple and/or isolated from other nodal areas of HD. Splenic involvement at splenectomy is designated by an ($_S$). The absence of constitutional symptoms is noted by an "A" subscript. The subscript "B" symptoms include unexplained weight loss of more than 10% of body weight within 6 months, unexplained fever, and night sweats. Pruritus and pain in involved areas following alcohol intake do not qualify as "B" symptoms.

Clinical Staging

A basic evaluation for extent of disease at presentation should include histologic confirmation of diagnosis, history and physical examination, liver biochemical studies, complete blood count, chest x-ray, and lymphangiogram unless contraindicated by previous history of allergy or restrictive lung disease.[139] Both serum copper and erythrocyte sedimentation rates fluctuate with disease activity but should not be considered reliable diagnostic parameters on which to base therapeutic decisions.

Whole-lung tomography or computerized axial tomography of the chest should be performed in those patients with bulky mediastinal masses and/or hilar involvement to rule out contiguous parenchymal lung involvement that might alter the radiotherapy portals. An intravenous pyelogram is not routinely indicated for the initial staging of HD unless the lymphangiogram shows the presence of bulky low para-aortic or pelvic nodes, in which case it might be useful to demonstrate ureteral obstruction and help in the design of the radiotherapy portal. Liver and spleen scan, skeletal survey, bone scan and gastrointestinal survey are not routinely indicated for the initial evaluation.

Computerized axial tomography of the abdomen gives reliable information regarding the presence of high para-aortic, celiac, porta hepatic, and mesenteric nodal involvement, not regularly demonstrated by lower extremity lymphangiogram.[38] Radioisotopic gallium scan appears to be much more accurate for the detection of HD in the neck and chest than in the abdomen.[113] It has been reported from various centers that the combination of gallium and CT scanning might have reliability similar to a lymphangiogram and have the additional advantage of potentially demonstrating high para-aortic nodal disease. However, our own experience does not confirm these conclusions. We have found gallium scan to be of relative usefulness only in the follow-up of mediastinal HD to distinguish it from radiation-induced mediastinal fibrosis.

When only clinical staging is planned, laparoscopy and guided liver biopsy[34,42] are most useful diagnostic tools to pursue the findings of abnormal liver chemistries (abnormal alkaline phosphatase and 5′-nucleotidase), positive lymphangiography, and/or suspected splenic involvement (clinical or radiologic evidence of splenomegaly or splenic defects). This indication is strengthened if constitutional "B" symptoms are present. Bone marrow aspiration and biopsy are probably not required for the initial staging of the common asymptomatic patient with stage I or II. However, they definitely should be performed upon clinical or pathologic evidence of stage III or IV disease and/or constitutional "B" symptoms.[137]

Due to its prognostic implications, HD in individuals more than 50 years of age deserves careful evaluation for the extent of disease and a close follow-up. Independent of the increased incidence of the lymphocyte deletion subtype in the elderly, there is a tendency for advanced stage, extranodal involvement, and poor tolerance and response to radiotherapy and/or chemotherapy.[104]

Pathologic Staging

According to the report of the Ann Arbor Committee, laparotomy and splenectomy for initial staging of HD should be performed only as a re-

search tool for populations in study, or if a therapeutic decision will depend on the identification of abdominal disease.[139] Staging laparotomy should include splenectomy and biopsy of suspicious para-aortic, celiac, porta hepatic, splenic hilar and/or iliac nodes, and liver and bone marrow from the iliac crest. It should be performed by surgeons well informed about the clinical indications and expectations of the procedure.[20]

Staging laparotomy studies have revealed that HD is often more widely disseminated than is first apparent.[4,40,57,91,123] The spleen is often involved when it is not evident clinically. Depending on the histologic type, unsuspected splenic involvement has been found in 20%–33% of patients upon careful examination of multiple tissue sections. In addition, the spleen is involved more often than the liver, and as the spleen size increases the incidence of liver involvement increases. Liver involvement almost never occurs without splenic involvement.[40,57,91] Patients with clinical stage III have approximately a 20% chance of having identifiable liver involvement with HD[4] and should therefore have operative examination of the liver by laparotomy or laparoscopy. Moreover, liver infiltration by HD is frequently associated with "B" symptoms and additional evidence of disease activity elsewhere. Histologically, it is characterized by lymphocytic infiltration of portal areas and requires the presence of typical or variant S-R cells[133] for confirmation. Similar histologic features are necessary in order to diagnose HD involvement in other extranodal sites. Of interest is the fact that sarcoidlike granulomas in liver and spleen have been associated with an improved survival in comparison to similar groups of patients without granulomas.[144]

Although it is not recommended as a necessary procedure for assessment of response to therapy, posttreatment laparotomy in HD patients has provided interesting information. Sutcliffe et al. reported that restaging laparotomy revealed persistent HD in 4 of 19 patients otherwise considered to be free of abdominal disease after chemotherapy, and the spleen was the most frequently involved site.[167] These findings argue in favor of systematic clinical restaging in all patients upon completion of their particular treatment programs, with the provision of pathologic evaluation for residual HD in the occasional suspected patient.

The combined data from several series show the morbidity from laparotomy averages 11% and ranges from 7% to 44%.[16,47,114,123,158] Mortality is approximately 1.5% (range 0–7%).[16,47,114,123] The risk of bacterial sepsis by either *Streptococcus pneumoniae* (pneumococcus) or *Hemophilus influenzae* appears to be significant, especially in children following splenectomy. In one laparotomy series, the risk of serious and sometimes fatal infection in lymphoma patients did not exceed the risk following splenectomy for other reasons.[149] However, this experience was different from that of other investigators,[156] the risk has been reported to be as high as 10% in children[31] and 20% in adult patients[177] treated for HD with irradiation and/or chemotherapy. The ability of the pneumococcal vaccine to prevent overwhelming postsplenectomy infection is doubtful. The currently available vaccine contains only 14 different polysaccharide serotypes providing immunization for 80% of all pneumococcal infections.[26,151] Moreover, pneumococcal vaccination has been most frequently administered after staging laparotomy and splenectomy and the poorest responders were the patients receiving intensive chemotherapy and radiotherapy.[155] At the present time pneumococcal vaccination appears most helpful in young patients prior to splenectomy and initiation of therapy.[101] The prophylactic administration of penicillin following splenectomy has been limited to the pediatric population.[31]

In regard to the possible advantages to be obtained by performing a splenectomy—such as reduction in the radiotherapy field size decreasing the risk of renal damage and radiation injury to a small portion of the left lung—it should be noted that these radiation effects can be avoided by shaping the splenic field by means of computerized axial tomography.[127] Oophoropexy with transposition of the ovaries, laterally or to the midline behind the uterus, should be part of the laparotomy procedure in young female patients.[169]

Therapy

Stages I–II

The significant improvement in the prognosis of the early stages of HD has resulted from the continuous progress in the development of radiotherapeutic techniques, contributed to by Gilbert,[56] Kaplan,[86,87] and Peters,[126] among others. The administration of tumoricidal doses of radiotherapy

by megavoltage beam equipment to fields encompassing the involved, adjacent, and transdiaphragmatic nodal areas at high risk for relapse has favorably influenced the remission rate and duration as well as survival.

Several randomized series of surgically[138] and clinically[81,84,90] evaluated patients with stages I and II have been reported, in which limited radiotherapy compared unfavorably to more extensive radiotherapeutic approaches with regard to disease-free survival (DFS). However, differences in survival have been more difficult to prove. Survival analysis at 10 years of the patients included in the National HD Study failed to reveal any differences between the groups treated with involved-field (IF) and extended-field (EF) radiotherapy (71% versus 75%, respectively).[82] Patients assigned to the IF program received treatment to include all areas of known disease plus a margin. EF included a mantle (mediastinal, hilar, supraclavicular, infraclavicular, cervical, and axillary node chains) (Figures 1–1 and 1–2) plus para-aortic and splenic fields for all patients with upper torso disease. An inverted Y port encompassing spleen or splenic pedicle, para-aortic, iliac, inguinal, and femoral node chains was used for patients presenting with disease in the lower torso (Figures 1–3 and 1–4). Patients with involvement of para-aortic nodes and spleen were treated with inverted Y plus mantle fields. A dose of at least 3500 rads in 3–4 weeks was given to all prescribed fields.

Preliminary analysis of 146 patients treated at Memorial Hospital (M.H.), New York City, with three different therapeutic modalities (IF, EF, or IF radiotherapy "sandwiched" between six cycles of MOPP chemotherapy) for stages I and II HD (Table 1–2) showed a significant improvement in the duration of remission free of any local or distant extensions achieved by a combination of IF

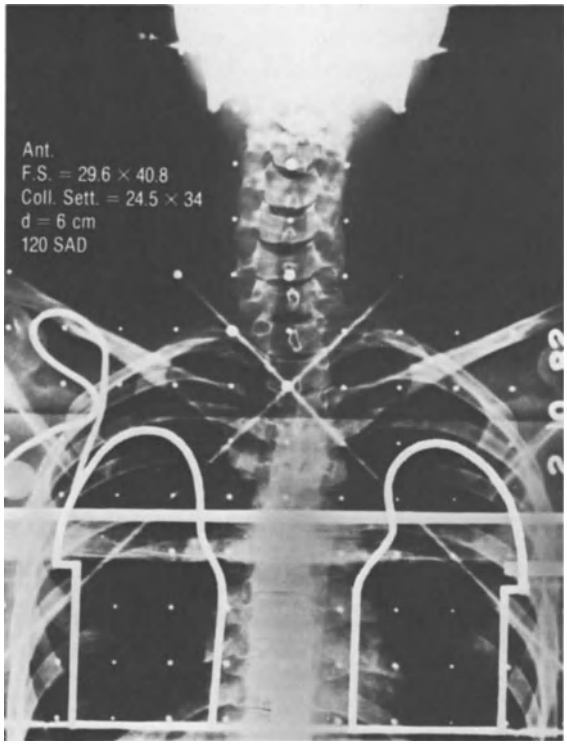

FIGURE 1–2 Simulator film to design the shielding for mantle portal.

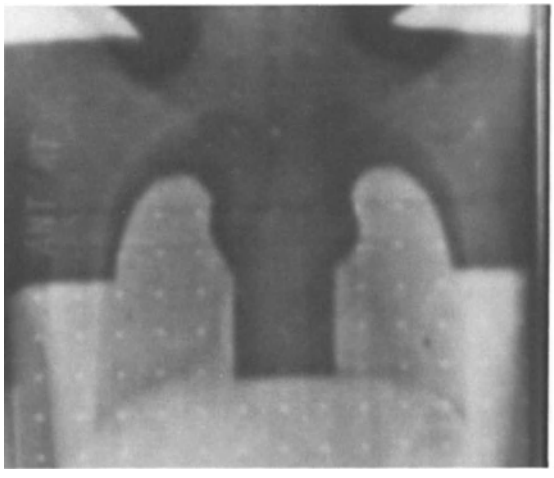

FIGURE 1–1 Beam film of mantle portal setup.

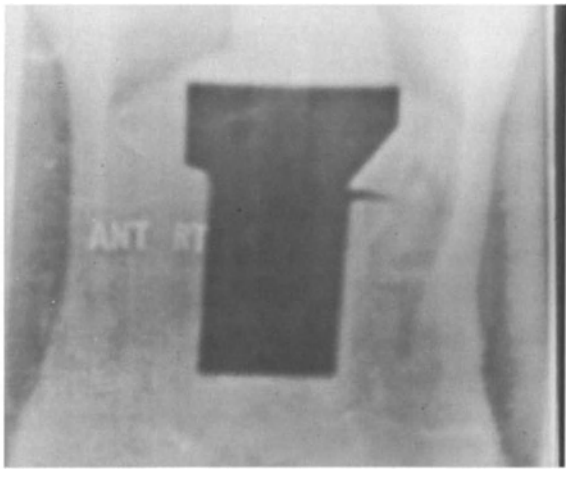

FIGURE 1–3 Beam film of upper para-aortic, splenic pedicle, and porta hepatic nodes portal.

FIGURE 1–4 Portal design for the inverted Y.

TABLE 1–2 Characteristics of the Patient Population

Characteristic	IF RT	EF RT	MOPP + IF RT
Total patients	46	46	76
Evaluable patients	40	40	66
Sex			
Male	20	18	32
Female	20	22	34
Median age (years)	25	24	29
Histology			
Nodular sclerosis	32	24	46
Mixed cellularity	7	14	12
Lymphocyte predominance	1	1	7
Not subclassified	—	1	1
Laparotomy			
Yes	28	26	14
No	12	14	52
Stage			
IA	7	12	20
IA_E (lung)	—	—	1
IIA	27	23	39
IIA_E (lung)	—	—	3
IIB	6	5	3

Abbreviations: IF RT and EF RT, involved-field and extended-field radiotherapy, respectively; MOPP, nitrogen mustard, vincristine, procarbazine, and prednisone.

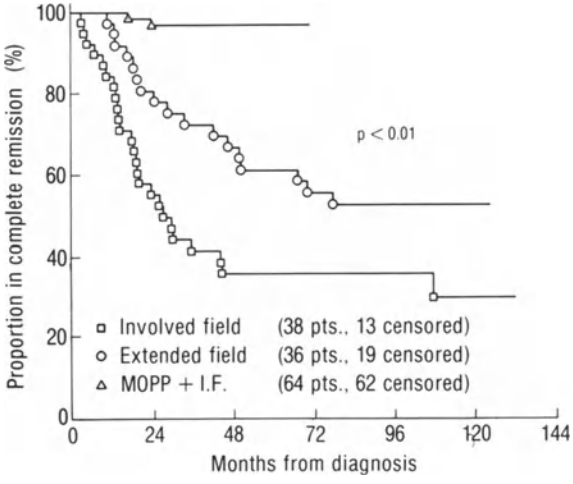

FIGURE 1–5 Disease-free survival of patients with stages I and II treated with three different modalities.

irradiation and MOPP chemotherapy (Figure 1–5). However, no differences on the overall survival have been observed up to the present. The IF and EF groups were treated from 1969 to 1973 within the randomized National HD Study. MOPP plus IF therapy was begun subsequently (1975–1979) as a single-arm trial.[35,96]

Analysis of the relapses observed in our group of patients[96] revealed that MOPP plus IF was successful in preventing distant transdiaphragmatic relapses in all except 1 of the 66 evaluable cases (Table 1–3). Although median follow-up time for this group has been only 42 months, the median time to relapse has been approximately 18 months. Despite the different follow-up times, the combination of MOPP chemotherapy and IF radiotherapy resulted in a significant decrease in the relapse rate compared with radiotherapy alone. Furthermore, IF radiotherapy compared unfavorably to EF radiotherapy in regard to relapse rate (mainly in nonsurgically staged and stage IA patients), the frequency of extranodal extensions, and the number of patients who are alive with recurrent HD or have died from HD.

This study has also shown that in a comparable group of patients, the performance of staging laparotomy followed by EF was an inferior program to clinical staging alone followed by MOPP plus

TABLE 1–3 Analysis of Relapses

Nature of Relapse	IF RT		EF RT		MOPP + IF RT	
	No.	%	No.	%	No.	%
Relapses from						
CR	23/38	61	17/36	47	2/64	3
PR	2/2	(1 died)	4/4	(3 died)	2/2	(2 IIAE died)
Median follow-up (months)	96		102			42
Median relapse time (months)	15	(2–106)	24	(3–76)	18	(12–24)
Laparotomy						
Yes	15/27	55	12/25	48	0/13	0
No	8/11	72	5/11	45	2/51	4
Relapses in stages						
IA	5/7	71	3/12	25	1/21	5
IA_E	—	—	—	—	0/1	0
IIA	14/26	54	11/21	52	0/43	0
IIA_E	—	—	—	—	0/1	0
IIB	4/5	80	3/3	100	1/2	50
Site of relapse						
Nodal Regional	7		7		1	
Transdiaphragmatic	4		8		1	
Extranodal	12		2		—	
Status of CR relapsed						
Alive, no evident disease	11		14		2	
Alive with HD	7		3		—	
Died with HD	5		—		—	

Abbreviations: IF RT and EF RT, involved-field and extended-field radiotherapy; MOPP, nitrogen mustard, vincristine, procarbazine, and prednisone; CR, complete remission; PR, partial remission.

IF with regard to DFS (Figure 1–6). Although this conclusion is based on the comparison of two selected groups and should be confirmed in a proper randomized trial, it stresses the controversial role of surgical staging in determining the most appropriate therapy for patients with HD. Pertinent to this point are the 5-year results of the EORTC randomized study of splenectomy versus splenic irradiation in 30 patients with supradiaphragmatic clinical stages I and II HD.[170] All patients were treated similarly with mantle and paraaortic node irradiation despite the degree of involvement observed in the laparotomy plus splenectomy group. Both actuarial survival and DFS were similar for the splenic irradiation and splenectomy arms (90% and 62%–67%, respectively). However, 42% of those patients found to have splenic involvement in the splenectomy arm relapsed, compared with an incidence of only 17% for those without splenic involvement. The group of patients with splenic involvement also had a 2-fold increase in the incidence of extranodal relapses and a 15-fold increase in the incidence of extranodal relapses in nonirradiated nodal areas (iliac and inguinal). These observations confirm the need for surgical staging for clinical stages I and II when only radiotherapy will be administered. However, in the same study, the 5-year relapse rate was only 11% for patients with mixed

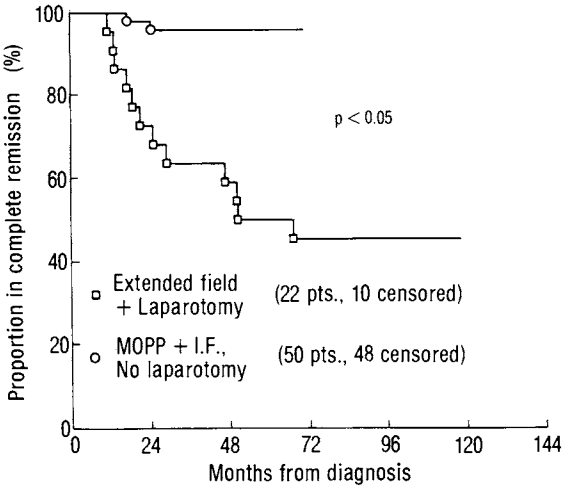

FIGURE 1–6 Disease-free survival of patients with stages I and II: comparison of surgical staging plus radiotherapy versus clinical staging plus combined chemotherapy and radiotherapy.

cellularity or lymphocyte deletion histologic subtypes. This low relapse rate was due to the prolonged administration of adjuvant chemotherapy with vinblastine alone or combined with procarbazine for supposedly less favorable disease, eliminating the advantage obtained by splenectomy.

The lack of an obvious survival gain following irradiation plus chemotherapy despite obvious differences in DFS appears to be due to the effectiveness of current salvage therapy of relapses following radiotherapy with further irradiation and/or MOPP, as observed in our series. Portlock et al. reported for the Stanford group an actuarial estimate of DFS of 40% at 5 years for the entire group of patients in first relapse, with a median of 25 months after salvage therapy.[129] However, the median survival of those patients relapsing after radiotherapy plus chemotherapy was 33 months, comparing unfavorably with the group treated with radiotherapy alone, in which 58% of the patients remained alive at 5 years after relapse.

In the most recent update of the Stanford experience, Hoppe et al.[78] reported that the 5-year DFS and survival among 230 patients with pathologic stages I and II were 79% and 96%, respectively, for radiotherapy alone (total or subtotal lymphoid irradiation according to histology) group, and 87% and 92%, respectively, for the radiotherapy (IF for I–IIA, subtotal lymphoid for I–IIA, IIA_E, or total lymphoid irradiation for I–IIB, IIB_E) plus chemotherapy (MOPP) groups.[79] The presence of extensive mediastinal involvement (mediastinal mass ratio more than one-third of the intrathoracic diameter) was the only significant predictor of HD relapse that was not confined to the chest. Histologic subtyping, systemic symptoms, and limited extranodal infiltration were found not to be significant prognostic factors. These observations contrast markedly with our own experience, since 8 of 10 cases with stage II_B (including 1 of 2 cases treated with combined modality) and 2 of 4 cases with substage "E" (lung) that achieved only a partial remission with MOPP plus IF radiotherapy have recurred with HD. It should be recognized that the radiotherapy fields and dosage used at Stanford for stages I and II (subtotal or total lymphoid irradiation including the ipsilateral lung upon hilar involvement) reflect an aggressive therapeutic approach for this pathologically staged population.

Distinct from the Stanford experience, Wiernik et al. compared EF versus EF plus MOPP and found a significant survival gain at 8 years in those patients treated with the combined modality approach.[180] At a median follow-up time of 69+ months from completion of therapy, 9 of 29 (31%) patients with stages I and II treated with EF alone had relapsed and 5 had died (18%). In contrast, only 1 of 17 (6%) patients with stages I and II treated with the combined modality had relapsed and there were no deaths caused by HD. Only 2 instances of acute myelomonocytic leukemia were observed, one at 48 months after EF radiotherapy and the other at 45 months after EF plus MOPP. In this randomized study, remission durations for EF and EF plus MOPP were comparable to ours for EF and IF plus MOPP.

Nissen et al. reported the results of a large, prospectively randomized study for the Danish National Hodgkin's Study Group comparing total nodal irradiation alone versus mantle radiotherapy followed by six cycles of MOPP for pathologic stages I and II with supradiaphragmatic HD.[122] At a median follow-up time of 4 years, significant differences in DFS were observed (97% versus 84%) favoring the use of the combined modality, although overall survival was similar for both groups. Most pertinent to the analysis of our treatment groups is the study carried out by the Southwest Oncology Group in patients with pathologic stages I and II, A or B, comparing in a randomized fashion EF versus IF followed by six cycles of MOPP.[36] MOPP plus IF was again significantly better than EF with regard to DFS in the entire population as well as when particular subsets of patients were analyzed separately (B symptoms, stage II, II_E, II_B, mediastinal presentation), although overall survival was similar in both groups of patients. However, in the Southwest Oncology Group trial, both EF and MOPP plus IF resulted in DFS that compared unfavorably with other series[79,180] and our own experience.

The possibility that less intensive adjunct chemotherapy may achieve similar results deserves further investigation. Improved duration of remission and survival in stages I and II have been reported by Hancock and Ledlie[71] with the use of a single dose of nitrogen mustard following EF irradiation. A similar advantage in DFS and survival was reported by the EORTC for a large randomized study involving the administration of 24 months of weekly vinblastine following 4000 rads EF irradiation.[45]

Up to the present time, no instances of secondary leukemia have been observed among our patients. However, one patient with stage I, mixed cellularity type, has developed a diffuse poorly differentiated lymphocytic non-Hodgkin's lymphoma. It is hoped that the limited radiotherapy administered to our patients in conjunction with MOPP will result in less leukemia compared to the experience reported by the Stanford group, which employed more extensive radiotherapy.[33,97]

Stage IIIA

From 1969 to 1974, the management of previously untreated stage III patients at Memorial Hospital was primarily a total nodal irradiation by split course and sequential radiation ("three and two") technique (Figure 1–7).[121] During the initial phase, radiotherapy (2000 rads in 4½ weeks) was administered to all lymphoid areas, which were divided into three segments: mantle, para-aortic, and pelvis plus inguinofemoral areas. After a rest period of 3–4 weeks, the same total nodal field was divided in two encompassing segments and irradiated to 1800–2000 rads in 3½ weeks. This technique minimizes the risk of overdosage at the junction of the portals and reduces the radiotherapy fields due to disease shrinkage during the rest period. The incidences of radiation-related pericarditis (2%), pneumonitis (0%), and hypothyroidism (3%) were markedly reduced compared with other radiotherapy designs.[132] All 26 patients with stage IIIA treated in this manner achieved a complete response. There were only 4 relapses, all occurring in extranodal sites within the first 12 months following total nodal radiotherapy.[121]

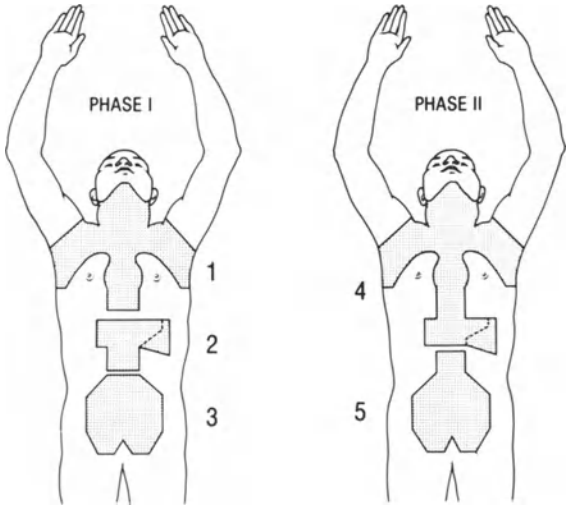

FIGURE 1–7 Phases I and II of the "three and two" technique for total nodal radiotherapy.

Since 1975, 20 patients with clinical (11 patients) or pathologic (9 patients) stage IIIA have been treated at Memorial Hospital with total nodal radiotherapy (total dose 2000 rads) "sandwiched" between six cycles of MOPP chemotherapy. Complete (CR) and partial remission (PR) rates were 84% and 16%, respectively. At 42 months median follow-up time, only 1 of the complete responders had relapsed and all patients were alive.

In recent years, subdivision of stage IIIA into two substages has been proposed to define prognostic groups that might require different treatment modalities. Stage $IIIA_1$ includes cases with involvement of spleen, splenic, celiac, or portal nodes, and stage $IIIA_2$ encompasses cases with involvement of para-aortic, iliac, or mesenteric nodes, with or without upper abdominal involvement. Stein et al., in a collaborative study involving 130 patients pathologically staged from three different institutions, found that both 5-year survival (94% versus 65%) and DFS (74% versus 46%) were significantly better for stage $IIIA_1$ than for $IIIA_2$, respectively.[161] Combined chemotherapy and radiotherapy was significantly superior to radiotherapy alone in prolonging DFS at 5 years in both stage $IIIA_1$ (96% versus 63%) and $IIIA_2$ (76% versus 32%). However, statistically significant overall survival differences comparing these two treatment modalities were evident only in the stage $IIIA_2$ group (84% for combined modality versus 56% for radiotherapy). For stage $IIIA_1$ (as similarly discussed for stages IA and IIA further follow-up might be required to detect any overall survival advantage from the use of combined therapy.

Stein et al. argued that combined chemotherapy and radiotherapy should be used for stage $IIIA_2$ but that radiotherapy alone would be sufficient for stage IIA_1.[161] This view was not shared by the Stanford group. Hoppe et al. failed to find prognostic differences for the two substages; however, they used more extensive radiotherapy (total lymphoid with supplemental radiation to liver).[78] Still, the Stanford investigators favored the use of combined therapy for patients with stage IIIA and diffuse splenic involvement and/or five or more involved nodal chains.

Several groups have reported on the use of che-

motherapy alone for stage IIIA. Although DeVita et al.[43] observed a 100% overall survival with the use of MOPP chemotherapy for a group of 23 patients with stages IIA, IIIA, and IVA, with only 1 relapse at 10 years, the British National Lymphoma Investigation[15] found in a randomized study that at 2 years, DFS for MOPP chemotherapy patients (53%) was significantly less than that of patients treated with total nodal radiotherapy (71%).

Patients with stage IIIB have more aggressive HD. With the use of total nodal radiotherapy, a median survival of only 32 months was noted by Aisenberg et al.[5] in 20 patients, and a 48% overall survival and 18% DFS at 8 years were reported by Rosenberg et al.[140] in 18 patients (who also received hepatic irradiation). Superior results have been observed with either chemotherapy alone[5,43] or combined modality treatment;[5,61,140] these are discussed with the treatment of stage IV.

Stages IIIB–IV

Few advances in cancer therapy have had as great an impact as MOPP combination chemotherapy has had on the prognosis of the advanced stages of HD. CR rates with MOPP and variants of MOPP have ranged between 47.5% and 80%. The latter result was achieved only by DeVita and coworkers in 198 patients with advanced HD (most of whom had stages IIIB and IVB).[41] The CR rate achieved at Memorial Hospital with MOPP was at the lower end of those reported in the literature. The 10-year follow-up from the National Cancer Institute group showed that 63.4% of the complete responders had remained in unmaintained remission from the end of treatment with an overall survival of 58%.[43] In that update, the presence of "B" symptoms, nodular sclerosis, and pleural involvement proved to be the main factors indicating a poor prognosis. The lack of effectiveness of maintenance chemotherapy in prolonging remission either with MOPP or bis-chloroethylnitrosourea (BCNU), initially reported by DeVita et al.,[41] has been confirmed by the Southwest Oncology Group.[35] Although Frei et al.[53] observed initial differences favoring the use of maintenance chemotherapy, further follow-up showed that at 7.2 years these differences were lost.[35]

Despite the remarkable results obtained with the use of MOPP, it should be pointed out at least 20% of patients with advanced disease will not achieve a CR and that more than 30% of the complete responders would have relapsed at 10 years. In an attempt to improve upon these results, a new treatment program using two different strategies was designed. The first was to add doxorubicin (Adriamycin), bleomycin, vinblastine, and dacarbazine (ABVD), a drug combination potentially non-cross-resistant with MOPP.[138] This regimen—which appears to be less leukemogenic[171] and less toxic to gonadal function than MOPP[145]—has been reported to be at least as effective as MOPP in inducing a CR (70% compared to 62%). Furthermore, upon primary treatment failure or relapse, crossover to the other regimen resulted in CR for two of five patients with ABVD and one of five with MOPP. The second was the use of adjunctive low-dose radiotherapy (2000 rads) to areas initially involved with bulky nodal disease greater than 5 cm, which would be likely sites of relapse after chemotherapy alone, as originally proposed by the Yale group.[50]

Between 1975 and 1978, a total of 118 patients with advanced HD were entered into the MOPP–ABDV–radiotherapy program at Memorial Hospital.[164] Sixty-seven were previously untreated; they had stage IIB, IIIA "unfavorable" (age greater than 35 years or mixed cellularity histology), IIIB, or IV HD. Patients were randomized to receive either levamisole 150 mg p.o. daily on days 1–6 and 15–20, or no treatment in the intervening periods between their bimonthly maintenance chemotherapy in months 10–24.

Among 57 evaluable previously untreated patients, 50 (88%) achieved a CR, 7 (12%) a PR, and no patient failed to respond. Among the 18 heavily pretreated patients, 9 (50%) achieved a CR, 4 (22%) a PR, and 5 (28%) failed to respond to treatment. The estimated relapse rate among previously untreated patients achieving a CR was 16% at 36 months from the date of remission. No differences in DFS or overall survival have been observed up to the present time between patients randomized to receive levamisole or no immunotherapy.

There have been two cases of acute leukemia, possibly secondary to treatment: one was in a patient who had relapsed after extensive abdominal and pelvic irradiation prior to initiation of MOPP–ABDV; the other was preceded by sideroblastic anemia in a previously untreated patient. Six patients had pulmonary complications, possibly related to bleomycin toxicity. None of the pulmo-

nary complications was fatal. One patient developed cardiomyopathy and heart failure, most likely related to the administration of doxorubicin. There were 35 deaths: 31 patients died of HD. Two died of second malignancies—one of acute leukemia and the other of carcinoma of the lung. One of the few patients who had a splenectomy as part of a staging laparotomy died of pneumococcal meningitis. Another died of well-documented graft-versus-host disease.

The 16% relapse rate among CR patients at 36 months compares very favorably with the 32%–65% relapse rates reported for MOPP alone.[43] Low-dose irradiation combined with chemotherapy seemed to be effective in achieving local disease control, since only one relapse occurred in an irradiated area among the previously untreated patients. A similar success in local control was reported by the Yale group,[50] who observed relapses in only 3 of 10 areas treated with low-dose irradiation (1500–2500 rads) in addition to a five-drug combination which added vinblastine to the drugs

TABLE 1–4 Salvage Combination Chemotherapy Regimens

Regimen	Drug	Dosage (mg/m^2)	Route	Schedule (days)	Response Rate (%)		Investigators
					Complete	Partial	
MOPP	Nitrogen						
(28-day cycle)	Mustard	6	i.v.	1,8	59	—	Fisher et al.[51]
	Vincristine	1.4	i.v.	1,8			
	Procarbazine	100	p.o.	1–14			
	Prednisone	40	p.o.	1–14			
ABVD	Doxorubicin	25	i.v.	1,15	60	—	Bonnadona et al.[12]
(28-day cycle)	Bleomycin	10	i.v.	1,15			
	Vinblastine	6	i.v.	1,15			
	DTIC	375	i.v.	1,15			
ABDV	Doxorubicin	25	i.v.	1,15	4	57	Case et al.[124]
(28-day cycle)	Bleomycin	2 (total)	s.c.	4–12, 18–26			Straus et al.[166]
	DTIC	250	i.v.	1,15			
	Vinblastine	6	i.v.	1,15			
SCAB	Streptozotocin	500	i.v.	1–5	35	24	Levi et al.[100]
(42-day cycle)	CCNU	100	p.o.	1			
	Doxorubicin	45	i.v.	1			
	Bleomycin	15	i.m.	1,8			
B-CAVE	Bleomycin	5	i.v.	1,28,35	50	27	Porzig et al.[131]
(42-day cycle)	CCNU	100	p.o.	1			
	Doxorubicin	60	i.v.	1			
	Vinblastine	5	i.v.	1			
B-DOPA	Bleomycin	4	i.v.	2,5	60	20	Lokish et al.[105]
(28-day cycle)	DTIC	150	i.v.	1–5			
	Vincristine	1.5	i.v.	1,5			
	Prednisone	40	p.o.	1–6			
	Doxorubicin	60	i.v.	1			
BVDS	Bleomycin	5	i.v.	1,15	30	20	Vinciguerra,[174]
(28-day cycle)	Vinblastine	6	i.v.	1,15			
	Doxorubicin	30	i.v.	1			
	Streptozotocin	1,500	i.v.	1,15			
CVB	CCNU	100	p.o.	1	26	59	Goldman and
(42-day cycle)	Vinblastine	6	i.v.	1,8			Dawson[160]
	Bleomycin	15	i.m.	1,8			
CAD	CCNU	100	p.o.	1	13	33	Straus et al.[165]
(28-day cycle)	Melphalon	6	p.o.	1–4			
	Vindesine	3	i.v.	1,8			

CCNU (lomustine). Chlorethylcyclohexyl nitrosourea: DTIC bric (dacarbazine Dimethyltriazeno-imidazole carboxamide)

used in MOPP. They reported an 84% CR rate for 124 patients, with a 12% relapse rate at a median follow-up time of 5 years. Two patients developed acute leukemia.

The Milan group has conducted a randomized trial of MOPP alternating with ABDV versus MOPP alone, with no adjuvant radiation therapy, for patients with stage IV HD.[146] Their results at 5 years showed a 71% CR rate with a 37% DFS for MOPP alone, compared to a 92% CR rate with a 70% DFS for MOPP–ABDV. Moreover, the 5-year survival difference was significant (84% versus 54%), favoring MOPP–ABVD over MOPP alone. Although three patients in the MOPP arm developed acute leukemia, this complication was not observed in the MOPP–ABVD group.

Another innovation in the design of combination chemotherapy–radiotherapy regimens was introduced by Hoppe et al.[77] The Stanford investigators compared two different combination chemotherapy regimens—MOPP versus procarbazine, melphalan (Alkeran), and vinblastine (PAV)—alternated with total lymphoid radiotherapy for 25 patients with stages IIB, IIIB, and $IIIB_S$. Total lymphoid irradiation was either sandwiched in a split-course fashion between the six cycles of chemotherapy or alternated between every two cycles of chemotherapy upon division of the total field in three regions ("ping-pong" technique). The latter design resulted in better hematologic tolerance. The CR rate was 88% (22 of 25), and 20 patients remained free of disease at a median of 28 months.

The efficacy of salvage chemotherapy for those patients with advanced HD relapsing after MOPP chemotherapy is dependent on several prognostic factors: One factor is the period of time between the initial response to MOPP and relapse. As reported by Fisher et al.,[51] MOPP reinduced a CR in a considerable proportion of patients (14 of 15) that relapsed more than 1 year after the initial remission. The likelihood of a second CR is minimal if the disease-free interval is less than 1 year, or if continuous evidence of disease activity is documented. The same observation applies to other salvage regimens. Another factor is whether the relapse is nodal or extranodal. Salvage chemotherapy is undoubtedly less effective when the recurrence takes place in major extranodal sites (such as bone marrow, liver, or bone) rather than being confined to lymph nodes.[166] The third factor is whether patients are treated in their first relapse or after multiple relapses.

The efficacy of ABVD as salvage chemotherapy has varied from 4% to 62%.[12,24] Moreover, several other salvage regimens have been tried with variable results, in all likelihood depending on the abovementioned prognostic factors. B-DOPA,[105] SCAB,[100] BVDS,[172] B-CAVE,[131] and CVB[60] represent alternative regimens to be considered in relapsing patients, as shown in Table 1–4. Vindesine has shown activity in relapsed HD patients who received prior vincristine and vinblastine.[157] Chloroethylcyclohexyl nitrosourea (CCNU), melphalan, and vindesine (CAD) was also active among prior MOPP or MOPP–ABVD treated relapsed HD patients.[165] Although a variety of new anticancer agents have been tried in relapsed HD cases (methyl GAG (Methyl-Glyoxal Bis-Guanylhydrazone),[174] gallium nitrate, interferon), they have failed to provide consistent and prolonged responses. Occasionally, the use of bleomycin given intravenously in constant infusion and high dose (20 units/m^2) for 5–7 days has resulted in dramatic although short-lived responses. Adequate pulmonary function should be documented prior to bleomycin therapy.

Current Treatment Trials at Memorial Sloan-Kettering Cancer Center

Although a longer follow-up time will be required to determine the impact of combined modality therapy for stages I, II, and IIIA on overall survival, gonadal function, and development of second malignancies, the combined use of MOPP chemotherapy and IF radiotherapy appears to be a superior regimen that might eventually result in a survival gain. It also avoids the necessity of surgical staging.[96] Future trials to determine the optimal management of patients with the localized stages of HD should compare the effectiveness as well as the toxicity of alternative regimens to MOPP as adjunct chemotherapy. Our current therapeutic protocol for stages IA, IIA, and IIIA consists of a randomized comparison of thiotepa, vinblastine and bleomycin, (TVB) with MOPP; both are given for four cycles with radiotherapy to segmental ports (3500 rads to mantle or inverted Y for stages IA and IIA, and 2000 rads to both ports for stage IIIA) "sandwiched" in between the second and third chemotherapy cycles (Table 1–5). A second randomization tests the efficacy

TABLE 1–5 Regimens for Randomized Comparison of Adjuvant Chemotherapy (MOPP versus TVB) Plus Segmental Radiotherapy

Regimen I: MOPP (2 monthly cycles)	
Nitrogen mustard	6 mg/m² i.v. days 1,8
Vincristine	1.4 mg/m² i.v. days 1,8 (maximum 2 mg)
Procarbazine	100 mg/m² p.o. days 1–14
Prednisone	40 mg/m² p.o. days 1–14 (only in first cycle)
Regimen II: TVB (2 monthly cycles)	
Thiotepa	35 mg/m² i.v. days 1,14
Vinblastine	6 mg/m² i.v. days 1,14 (maximum 10 mg)
Bleomycin	2 mg s.c. days 4–12, 18–26
Radiotherapy ("sandwiched" between chemotherapy cycles 2 and 3)	
Segmental ports to involved areas:	
Mantle	
Para-aortic nodes and spleen	
Iliac nodes	
Dose:	
3500 rads in 3½ weeks in stages I–II	
2000 rads in stage III	

of hormonal manipulation (androgens for male and contraceptives for female patients) for the prevention of gonadal disturbances due to this combined modality of therapy.

Based on the marked improvement in CR rate and DFS observed with the use of MOPP–ABVD plus radiotherapy in our prior trial[164] and the encouraging effectiveness of CAD as salvage chemotherapy,[152] a randomized trial of MOPP–ABVD versus MOPP–ABV–CAD was begun in 1979 to include previously untreated patients with stages IIB, IIIB, IVA, and IVB HD (Figure 1–8). Adjunctive radiotherapy is part of both arms of the randomization. The dose is 2000 rads delivered to involved regions in 2½ weeks. In the new regimen, MOPP–ABV–CAD plus radiotherapy, the dacarbazine was dropped from ABVD to reduce nausea and vomiting, and CAD is alternated with MOPP and ABV as a third non-cross-resistant combination in an attempt to improve our results with MOPP–ABVD. Nine alternating cycles of MOPP–ABVD or MOPP–ABV–CAD are administered with radiotherapy given between cycles 6 and 7. Thus far, remission rates with MOPP–ABV–CAD plus radiotherapy are at least as high as those with MOPP–ABVD plus radiotherapy, and patient acceptance has been greatly improved with the reduction in nausea and vomiting.

Complications Related to Therapy

The progressive improvement in the management of HD has unfortunately also caused long-term side effects, the most serious of which is the development of secondary neoplasia. Several mechanisms have been suggested to explain the high incidence of secondary malignancy in HD. It has been proposed that the hematologic neoplasias that occur after therapy for HD represent malignant progression of the original primary disease. It has also been suggested that the leukemia represents the underlying neoplasm and that HD results from a persistent atypical immunologic reaction on the part of the patient.[70] According to the Stanford group's experience, the cumulative risk for the development of secondary acute leukemia following combined modality therapy for HD was in excess of 5%. Moreover, for the same patient population they also reported that the actuarial risk of developing secondary non-Hodgkin's lymphoma at 10 years was 10%–15%.[88] However, despite these previous estimates, at 6 years median follow-up, only 1 of the 121 stage I–II patients treated with combined modality has developed acute leukemia.[79]

At Memorial Hospital a multidisciplinary methodology was appied to study six patients with acute leukemia following treatment for HD.[63] All six patients developed acute monocytic leukemia according to cytochemical criteria; morphologically, three cases were undifferentiated monoblastic leukemia, and three cases were partially differentiated monocytic leukemia. In three of the six patients, cell suspensions from peripheral blood and/or bone marrow predominantly bore surface receptors for the Fc fragment of IgG and/or C3 fraction of complement and exhibited variable degrees of phagocytosis with later incubation. Two patients lacked any recognizable membrane markers or phagocytic activity. Terminal transferase enzymatic activity was not elevated. Soft agar bone marrow culture studies revealed no growth or a characteristic leukemic cluster pattern. Chromosome analysis in one of the two patients studied revealed hypodiploidy and structural arrangement.

Rosner and Grunwald reviewed the available literature up to 1974 on the development of leukemia in patients with HD.[141] It was noted that 11

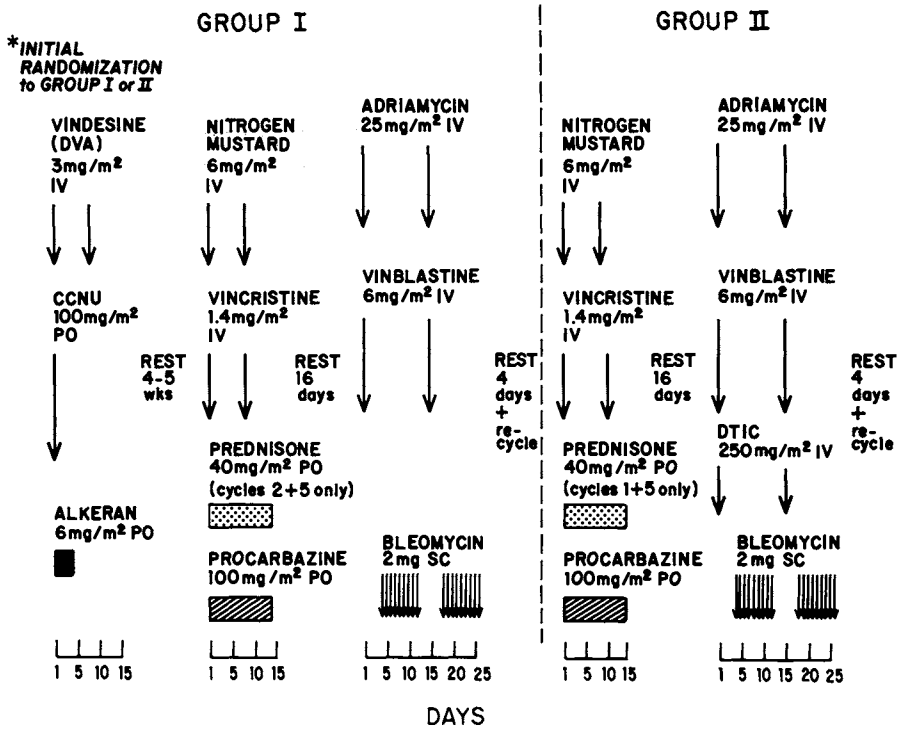

FIGURE 1–8 Description of MOPP–ABV–CAD versus MOPP–ABVD plus segmental radiotherapy protocol for advanced stages of HD.

cases of HD terminated in acute lymphoblastic leukemia, 12 in chronic myelogenous leukemia, 37 in chronic lymphocytic leukemia, and 82 in acute myelogenous leukemia. Further characterization of the 82 cases of acute myelogenous leukemia revealed 19 cases of acute myeloblastic leukemia, 11 cases of monoblastic or monocytic leukemia, 14 cases of myelomonocytic leukemia, 26 cases of partially differentiated or mature myeloid cell leukemia, 4 cases of erythroleukemia, and 8 unclassified cases.

In the retrospective analysis by Coleman et al., among 680 patients with HD studied, 6 patients developed leukemia while they were in clinical remission.[33] Three additional patients who had active HD also developed leukemia. Secondary neoplasia was not observed in 320 patients treated with radiotherapy alone. In our series, 4 patients had been treated with radiotherapy plus chemotherapy; 2 had received chemotherapy alone.[63] Despite the institution of appropriate chemotherapy, all of our 6 patients who developed leukemia died, and the median survival was only 3 months. We and others have observed that the incidence of acute monocytic leukemia is higher in patients receiving chemotherapy as salvage treatment upon relapse from primary irradiation, than in patients whose initial therapy is combined chemotherapy and radiotherapy.[7,171]

Immunosuppressed and immunodeficient patients are known to have a higher incidence of malignancies.[108] As previously discussed, cell-mediated immunity is depressed in many patients with HD, and this abnormality is accentuated by ionizing radiation and chemotherapy. Radiation to the mediastinum can depress the proportion of thymus-dependent (T) lymphocytes, which in turn leads to defective cell-mediated immunity.[185] Four of our six patients received radiation below and above the diaphragm, including the area of the mediastinum. Among the chemotherapy agents used in HD, the alkylating agents (nitrogen mustard, cyclophosphamide) and procarbazine appear to be the most carcinogenic.[72] It is postulated that mutations occurring in the cell due to the action of alkylating agents and/or radiotherapy lead to the production of an additional malignant clone, enhancement of mutagenesis by an oncogenic virus, or suppression of the body's mechanism of immune vigilance.

Chromosomal analysis of 10 patients with acute nonlymphoblastic leukemia following treatment

for HD or non-Hodgin's lymphoma revealed that all 10 had abnormal karyotypes in the bone marrow cells, often in a hypodiploid state with 42–45 chromosomes.[142] Of the 10 patients, 9 lacked a group B chromosome, which could be identified as chromosome 5 in 8 cases; 5 of 10 lacked chromosome 7. Two patients in our series had cytogenetic studies.[63] In one, 9 of 11 cells examined were abnormal; hypodiploid cells with many structural rearrangements of the chromosomes were noted. The second patient revealed only normal metaphases. Although chemotherapy and radiotherapy might be expected to contribute to the development of chromosomal aberrations, the early finding of chromosomal abnormalities could be an indication of the eventual development of leukemia.

Intermediate-dose radiotherapy (3500–4500 rads) to cervical ports in HD patients has been associated with a high incidence of hypothyroidism (20%)[54] and thyroid dysfunction, manifested as elevated thyroid-stimulating hormone levels (44%–64%), with or without subnormal thyroid levels.[163,164] The experimental evidence of an association between elevated thyroid-stimulating hormone level and neoplasia raises concern about the possible development of thyroid carcinoma in patients treated with cervical radiotherapy for HD after prolonged follow-up (20–30 years). The combination of radiotherapy and lymphangiogram for initial staging has been blamed for the biochemical and, less frequently, clinical evidence of hypothyroidism, although recent evidence tends to bring this synergism into question.[120,153]

Also of concern is the late development of cardiovascular disease after mediastinal irradiation alone or in combination with anthracycline chemotherapy.[14] The incidence of radiation-induced chronic pericardial disease has been reported to range between 6% and 30%.[6,143,163] Furthermore, the increased recognition of radiation-related myocardial fibrosis and early coronary disease will require close monitoring of these patients to detect minimal functional defects.

Amenorrhea and azoospermia represent major complications of cytotoxic chemotherapy. Ovarian biopsies in patients who have received such therapy have shown widespread ovarian damage: thickening of the albuginea, perioophoritis, stromal fibrohyalinization, necrotic vasculitis, hemorrhage, and general disintegration of the follicular apparatus.[116] Testicular biopsies in patients receiving chemotherapy have shown peritubular fibrosis, germinal aplasia, and normal Leydig cells.[49] Hormonal measurements in patients developing amenorrhea and azoospermia on therapy have confirmed end-organ failure with associated elevations of follicle-stimulating hormone and luteinizing hormone levels. Although a decrease in estrogen levels in women has been noted, Leydig cell function in males has been found to be normal or depressed.

Recent reports have concurred that the chemotherapy-induced ovarian failure is age related. The National Cancer Institute group reported that although 46% of 24 MOPP-treated patients (median age 30 years) developed persistent amenorrhea, 80% of the patients younger than 25 years of age at the time of MOPP therapy continued to menstruate.[148] Horning et al., from Stanford, released a comprehensive study of menstrual status and pregnancy in 103 women with HD treated with total lymphoid irradiation, multidrug chemotherapy, or a combination of both modalities.[80] The median age of the total group was 23 years, distributed evenly among the three treatment groups. The incidences of persistent amenorrhea for the total lymphoid irradiation, chemotherapy, and combined treatment groups were 6%, 15%, and 52%, respectively. In a multivariate analysis, only age at treatment was a significantly consistent predictor of regular menses. Pregnancies occurred in 7 of the patients treated with MOPP at the National Cancer Institute[148] and in 20 of the patients treated with irradiation, chemotherapy, or both at Stanford.[80] Up to the time of both reports, the children born to these women were phenotypically normal.

Chapman et al., in evaluating 74 males with HD receiving a multidrug regimen of nitrogen mustard, vinblastine, procarbazine, and prednisone (MVPP), noted a strikingly high incidence of persistent germ cell dysfunction.[29] Only 4 of 74 males showed evidence of spermatogenesis. However, the median follow-up was 27 months with a range of 1–62 months. Sexual dysfunction was also noted in patients of both sexes.[28,29] Decreased libido was reported by 74% of the males in therapy and 16% after therapy. The same group carried out a prospective study in untreated patients with HD, and found that 33% of the patients investigated had "inadequate" sperm counts with regard to number and/or motility. Moreover, testi-

cular biopsies in 8 of 9 patients showed histologic abnormalities (mostly tubular hyalinization and thickening of the basement membrane).[30]

Due to the universal azoospermia that occurs promptly after administration of chemotherapy, storage of sperm prior to any therapeutic intervention should be routinely proposed to those patients wishing to have children. Whitehead et al. proposed that contraception is unnecessary from the second cycle of combination chemotherapy (MVPP) until the end of the first year after completion of chemotherapy.[179] This should be followed by an analysis of sperm counts every 6–12 months to determine further conduct. In available studies, the actual degree of reversibility of gonadal damage and time to such gonadal recovery is not well established. After the administration of cyclophosphamide, Buchanan et al. found a 50% recovery of spermatogenesis within 15–49 months (mean 31 months).[19] In lymhoma patients evaluated retrospectively after therapy, Sherins and DeVita noted that 4 of 12 patients in remission at 2, 3½, 4, and 7 years had spermatogenesis.[154]

Recently, the use of hormonal manipulation has been proposed to prevent gonodal damage. Encouraging evidence has been reported about the protective effect oral contraceptives have on ovarian function in chemotherapy-treated HD patients.[27] Furthermore, the reversible inhibition of testicular steroidogenesis and spermatogenesis caused by a gonadotropin-releasing hormone agonist (LRHR) might have clinical application in HD patients.[103]

Concluding Remarks

It could be envisioned that during the coming years an outburst of new information will become available about the biology of HD based on the application of hybridoma technology. Specific monoclonal antibodies to the different mononuclear and multinucleate cellular components of HD will probably become available. These reagents will not only be useful in dissecting the cellular interactions involved in neoplastic development, but also as diagnostic and eventually therapeutic modalities.

Based on the information to be obtained from ongoing clinical trials, it is expected that the controversy in regard to the optimal therapy for the early stages of HD will be resolved. It is our belief that ultimately, surgical staging and extensive radiotherapy will be superceded by less toxic combined modalities of chemotherapy and radiotherapy. Furthermore, the treatment of the late stages of HD will most likely be based on the use of alternating, non-cross-resistant chemotherapy regimens.

The advances in the management and therapy of HD will guide the approach to other neoplastic conditions. It is hoped that this progress will be based on a rational understanding of the biology of HD—a goal that thus far has been elusive to the students of this most challenging process.

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CHAPTER 2

Non-Hodgkin's Lymphoma

David J. Wolf

Of the approximately 30,000 new patients diagnosed to have lymphoma in the United States during 1980, approximately one-fourth had Hodgkin's disease. The remaining three-fourths were diagnosed to have a variety of different types of lymphoma, collectively categorized as non-Hodgkin's lymphoma. The average age of these 22,500 people was 42 years. The incidence of non-Hodgkin's lymphoma in the United States is increasing annually. The age distribution shows a steady rise from adolescence through the ninth decade of life. Males are affected approximately 1.5 times more often than females.

The non-Hodgkin's lymphoma affecting adults in the United States is considered here, with the exclusion of American Burkitt's lymphoma and cutaneous T cell lymphoma, which are considered elsewhere. Basic concepts regarding the etiology, histopathologic classification, and treatment are currently active areas of both biologic and clinical research. New varieties of lymphoma are constantly being recognized, such as a T cell lymphoma involving the skin and viscera described in Japan, and a very aggressive lymphoma having a predilection for the central nervous system seen among the immunodeficient homosexual male population in the United States.[52] Nevertheless, therapeutic strides have been made over the past decade, particularly in the management of advanced, diffuse large cell lymphoma in adults, for which potential cures are now being achieved.

Etiology and Pathogenesis

Viruses cause lymphomas in animals. A type C retrovirus can transmit bovine lymphocytic lymphoma in a highly infectious manner.[54] A lymphoma of chickens, called a Marek's disease, is caused by a herpes virus and can be prevented by vaccination.[24] A viral causation for human lymphomas, however, remains to be established. Although Epstein-Barr virus B DNA has been shown to be incorporated into the DNA of Burkitt's lymphoma cells derived from African patients, this is not necessarily true in the American form of Burkitt's lymphoma.[41] Gallo et al. recently demonstrated a type C retrovirus as a possible cause for T cell human lymphoma.[16] Direct transmission of lymphoma in humans by a viral particle has never been demonstrated and probably never will, owing to ethical considerations.

The currently identified predisposing factors to developing lymphoma in humans are listed in Table 2–1. Exposure to ionizing radiation can cause lymphoma in humans. Survivors of the atomic bomb in Hiroshima, exposed to 100 rads or more, have demonstrated an increased incidence of lymphoma.[34] This has also occurred in patients who have been irradiated for ankylosing spondylitis.

Derangements of normal immunologic mechanisms also predispose to lymphoma. Patients who have either underlying immunodeficiency states or hyperimmune states treated with immunosuppressant programs are at particular risk to develop malignant lymphoma. Congenital immunodeficiency diseases, such as acquired hypogammaglobulinemia, common variable immunodeficiency disease, and the Wiskott-Aldrich syndrome, confer an increased risk of malignant lymphoma.[32] Renal allograft recipients are at a 35 times greater than normal risk of developing malignant lymphoma.[39] Approximately 40% of such lymphomas complicating renal allograft and immunosuppressive therapy occur in the central nervous system and

TABLE 2–1 Predisposing Factors to Develop Malignant Lymphoma

Congenital Immunodeficiency Syndromes With or Without Chromosomal Abnormalities
Ataxia telangiectasia syndrome
Chediak-Higashi syndrome
Common variable immumodeficiency disease
Klinefelter's syndrome
Swiss-type agammaglobulinemia
Wiskott-Aldrich syndrome
Acquired Immunoregulatory Disorders
Acquired hypogammaglobulinemia
Angioimmunoblastic lymphadenopathy
Celiac disease
Chronic lymphocytic leukemia or Waldenström's macroglobulinemia (Richter's syndrome)
Cytoxic chemotherapeutic agents
Ionizing radiation
Male homosexuality
Phenytoin administration
Renal allograft transplantation
Sjogren's syndrome

are most often immunoblastic B cell lymphomas.[30]

Phenytoin perturbs immunoregulatory mechanisms, and lymphomalike syndromes with generalized lymphadenopathy may occur. Although most often the lymphadenopathy regresses following phenytoin discontinuation, the emergence of both non-Hodgkin's and Hodgkin's lymphomas has been described.[18] Cytotoxic and immunosuppressive chemotherapeutic agents may cause chromosomal abnormalities and confer an increased risk of developing lymphoma. Non-Hodgkin's lymphoma has been reported to complicate combined modality therapy, consisting of combination chemotherapy and radiotherapy for the treatment of Hodgkin's disease and other malignancies.[30] Angioimmunoblastic lymphadenopathy is a disease characterized by overactivity of the B cell arm of the immune system. Immunoblastic lymphoma composed of B cells may develop in patients suffering from angioblastic lymphadenopathy.[5a] Sjögren's syndrome of longstanding duration confers up to a 10% chance of developing malignant lymphoma, usually nodal and composed of malignant B lymphocytes.[25] The reason only particular patients with such underlying predispositions develop lymphoma is unclear. Perhaps it is related to some inherited genetic susceptibility. An association between nodular lymphoma and HLA-B12 antigen has been reported.[11] For animal lymphomas, inbreeding and viruses play a synergistic role.

The development of the technique of chromosome banding with quinacrine and Giemsa staining in the early 1970s has revolutionized the study of human cytogenetics. The most frequently seen chromosomal abnormality in lymphomas is the 14q+ chromosome. This occurs in virtually every Burkitt's lymphoma and some non-Hodgkin's lymphomas.[43] It appears to occur more often in association with poorly differentiated lymphocytic lymphoma than in diffuse large cell lymphoma. It also occurs in approximately one-half of T cell malignant lymphomas. It remains unclear whether the particular donor chromosome has any biologic importance. With the exception of the 14q+ chromosome, other chromosomal abnormalities occur more frequently in diffuse large cell lymphoma than in diffuse, poorly differentiated lymphocytic lymphoma. The 14q+ abnormality, however, is not specific for lymphoma and has been reported to occur in patients with B cell acute lymphoblastic leukemia and with plasma cell myeloma.[28] It is very rarely, however, seen in myeloid malignancies.

For reasons that remain unclear, an epidemic of abnormal immunologic regulation is occurring among the male homosexual populations of New York City and San Francisco. Such immunosuppressed patients are commonly found to have evidence of infection by multiple viruses, including the DNA viruses, Epstein-Barr virus, and cytomegalovirus. A particularly aggressive form of B cell lymphoma with morphologic similarities to Burkitt's lymphoma is emerging in this particular population. The lymphoma shows extremely short-lived remissions following treatment with intensive combination chemotherapeutic programs and has a particular proclivity to involve the central nervous system.

Surface Markers in Non-Hodgkin's Lymphoma

Surface market studies allow the identification of the origin of the cell in non-Hodgkin's lymphoma.[10a] An immunological classification affords the clinician information that may be helpful in determining the kind of treatment and probable outcome.

B-lymphocytes are identified by surface membrane bound immunoglobulins consisting of a heavy chain and a light chain produced by the

cell. Immunofluorescence is used to identify the surface membrane markers.

Leukocyte antigens ("Ia-like") are useful to help identify B-cells, monocytes, macrophages, myeloid, and erythroid progentor cells, as well as "activated" T-cells.[11]

Additional identifying B-cell surface markers are receptors for (1) rosette formations with mouse erythrocytes, (2) Fc fragments of IgG that have been heat aggregated (EA), and (3) third fraction of complement using a rosette technique with erythrocytes (e) coated with IgM antibody (A) and complement (C) (EAC).[65c]

Although most histiocytes, macrophages, and monocytes have Fc and complement receptors, their phagocytic properties (ingestion of iron-filings, bacteria, latex-particles) help to differentiate them from B-lymphocytes. In addition, cytochemical staining employing non-specific exterases help in differentiating the phagocytic cells from the B-cells.

T-lymphocytes derived from the thymus form spontaneous rosettes with sheep erythrocytes; employing serum incubation in the cold or pre-treatment of the sheep erythrocytes with 2-amine-thyliso-thiuronium bromide (AET) or neuroaminidase will enhance the rosettes.[76c]

Clinical Presentation and Disease Evolution

Most patients with non-Hodgkin's lymphoma present with asymptomatic lymph node enlargement. Approximately 20% have symptoms related to their illnesses, such as fever, weight loss, or night sweats. In contrast to Hodgkin's disease, non-Hodgkin's lymphoma is rarely localized to one lymph node group or to contiguous lymph node groups. Although non-Hodgkin's lymphoma is presumably unifocal in origin, the disease remains localized so transiently that generalized disease is present at the time of diagnosis in approximately 90% of patients. Lymph nodes in the epitrochlear area, Waldeyer's ring, and the mesentery are involved much more often than in Hodgkin's disease. Extranodal involvement is also more common than in Hodgkin's disease, with the gastrointestinal tract, testes, bone marrow, and liver frequently involved. Although mediastinal lymph node enlargement occurs much more frequently in Hodgkin's disease, T cell lymphoblastic lymphoma characteristically presents with mediastinal enlargement during the second or third decade of life. Although patients with nodular lymphoma may apparently have localized adenopathy, generalized adenopathy is usually demonstrable by studies which may reveal hilar, retroperitoneal, or mesenteric lymph node enlargement. In general, diffuse non-Hodgkin's lymphomas tend to be more localized than nodular varieties. Up to 40% of patients with nodular lymphomas eventually transform to diffuse lymphomas.[21] Whether this actual evolution occurs independently of therapy remains unknown.

Particular clinical presentations are associated with specific varieties of non-Hodgkin's lymphomas. As mentioned above, a mediastinal mass suggests a T cell lymphoblastic lymphoma. Cranial nerve abnormalities or central nervous system dysfunction in general suggests lymphomatous meningitis as most commonly occurring in diffuse large cell lymphomas. Bulky generalized lymphadenopathy in an asymptomatic patient speaks for a nodular B cell lymphoma. Cutaneous nodules indicate the likelihood of a T cell lymphoma, such as described in Japan. This particular type of lym-

TABLE 2–2 Staging Procedures for Hodgkin's Disease and Non-Hodgkin's Lymphoma

Adequate Surgical Biopsy Interpreted by an Experienced Pathologist in Lymphomas. Marker Studies if Available.
History (in Hodgkin's disease)
Note: Presence or absence of weight loss, pruritis, fever, sweating—that is, "B" Sx.
Physical examination—inspection of Waldeyer's ring.
Note: Size, consistency of peripheral lymph nodes. Record size of liver and spleen.
Hematological Profile—CBC, exact platelets
Screening chemistries—SMA-12
Lactic dehydrogenase
Alkaline phosphatase
Bone marrow—aspiration and biopsy
Chest x-ray—PA and Lateral
Bilateral lower limb lymphangiogram
Intravenous pyelogram
Procedures Employed in Special Settings
Chest tomograms
Cat scan of chest and abdomen with pelvis
Ultrasonography
Hepato-splenic scintigrams
Gallium whole body scans

TABLE 2–3 B-Cell Associated Lymphoid Neoplasms in Adults

Diffuse well-differentiated lymphocytic lymphoma
Nodular and diffuse poorly differentiated lymphocytic lymphoma
Nodular mixed lymphocytic lymphoma
Nodular histiocytic lymphocytic lymphoma
Diffuse poorly differentiated lymphocytic lymphoma
Diffuse mixed lymphocytic-histiocytic lymphoma
Diffuse histiocytic lymphoma
Diffuse lymphoblastic lymphoma
Diffuse undifferentiated lymphocytic lymphoma
Burkitt's lymphoma
Richter's syndrome
Immunoblastic sarcoma

TABLE 2–4 T-Cell Associated Lymphoid Neoplasms

Acute lymphatic leukemias of children
Chronic myeloid leukemia—blast phase
Mycosis fungoids, Sezary syndrome
Chronic lymphatic leukemia—some patients
Diffuse "histiocytic" lymphoma—Rappaport

TABLE 2–5 Null Cell Associated Neoplasms in Adults

Acute lymphatic leukemia—children (most)
Lymphoblastic malignant lymphoma
Blast phase chronic myeloid leukemia
Diffuse histiocytic lymphoma (40%)—Rappaport

phoma is aggressive and often infiltrates viscera.[52] Malabsorption and gastrointestinal symptoms characterize the so-called Mediterranean lymphoma, which is a lymphoplasmacytic lymphoma often secreting alpha heavy chains. Immune hemolytic anemias caused by either warm- or cold-reactive anti–red cell antibodies, a monoclonal serum immunoglobulin, bone pain, or a chylous pleural effusion suggest a diagnosis of lymphoma in a patient with significant peripheral lymphadenopathy. (See Tables 2–2 through 2–5.)

Pathology

Although a complete discussion of the pathology of non-Hodgkin's lymphoma is beyond the scope of this chapter, consideration must be given to the present controversies regarding pathologic classification. The Rappaport classification devised in 1956 placed primary importance upon the presence or absence of a follicular or nodular nodal architecture, and secondary importance upon the predominant cell type. Owing to its simplicity and ability to provide clinically corroborated prognostication, it has found great favor among clinicians. Subsequent advances in the understanding of the immunologic nature of malignant lymphoid cell populations, however, has uncovered a major flaw in the Rappaport scheme. The cell that Rappaport called the histiocyte, as the predominant cell in histiocytic lymphoma, has been shown to represent a cell of lymphoid origin, and not histiocytic origin. Thus, if one replaces the term "large cell" for "histiocytic" lymphoma, the Rappaport classification remains, for the most part, clinically useful and simple. Nevertheless, it does fail to segregate certain varieties of lymphoma. The Rappaport classification is shown in Table 2–6.

Resulting from the development of new techniques in identifying populations of lymphoid cells, five additional, entirely different pathologic classifications have been proposed during the 1970s. A most popular classification in Europe has been the Kiel classification, proposed by Prof. Karl Lennert, in which the grade of malignancy is of primary consideration, with subcategories indicating the variety of immunotopography.

In an attempt to coordinate the six proposed pathologic classifications for the non-Hodgkin's lymphomas, the National Cancer Institute undertook a retrospective study of over 1000 cases of non-Hodgkin's lymphoma beginning in 1976. This undertaking has culminated in a working formulation, shown in Table 2–6. The intent of the formulation is not to create a new classification with diagnostic categories, but rather to provide a universal framework with which the contending classifications may be coded to a uniform nomenclature. Since the Rappaport classification provides good clinical correlation, it will be used primarily throughout the remainder of this chapter, with reference made to the working formulation nomenclature.

Nodular (Follicular) Lymphoma

The most common form of nodular lymphoma is the nodular, poorly differentiated lymphocytic type (NPDL), also known as the follicular, small cleaved cell type. It usually affects adults over the

TABLE 2–6 National Cancer Institute Working Formulation of Non-Hodgkin's Lymphomas with the Respective Rappaport Term for Each Histopathologic Type

Working Formulation	Rappaport Term
Low grade	
Small cell lymphocytic (SC)	Diffuse, well-differentiated lymphocytic (DWDL)
Follicular, predominantly small cleaved cell (FSC)	Nodular, poorly differentiated lymphocytic (NPDL)
Follicular, mixed small cleaved and large cell (FM)	Nodular mixed (NML)
Intermediate grade	
Follicular, predominantly large cell (FL)	Nodular histiocytic (NHL)
Diffuse, small cleaved cell (DSC)	Diffuse poorly differentiated lymphocytic (DPDL)
Diffuse, mixed small and large cell (DM)	Diffuse mixed (DML)
Diffuse, large cell (DL): cleaved or noncleaved cell	Diffuse histiocytic (DHL)
High grade	
Immunoblastic (IBL)	Diffuse histiocytic (DHL)
Lymphoblastic (LL): convoluted or nonconvoluted cell	Diffuse lymphoblastic (DLL)
Small noncleaved cell: Burkitt's or non-Burkitt's	Diffuse undifferentiated (DUL)
Miscellaneous	
Composite	
Mycosis fungoides	
Histiocytic	
Extramedullary plasmacytoma	
Unclassifiable	

age of 40 years and presents as asymptomatic, painless lymph node enlargement. Focal bone marrow involvement occurs in up to 85% of patients with NPDL and is perhaps even higher if light chain typing of marrow lymphocytes is performed. Involvement of the central nervous system, liver, lungs, and Waldeyer's ring, however, is unusual at presentation. The initial clinical course is indolent, with clinical stability or slight progression over a period of 5–7 years. Following a prolonged, indolent course, a transformation of the illness occurs, often characterized by rapidly enlarging lymph nodes, nonlymphoid organ infiltration, appearance of systemic symptoms, refractoriness to standard chemotherapeutic programs, and a metamorphosis of lymph node pathology in which a diffuse architectural pattern develops, as well as the appearance of larger and more poorly differentiated lymphoid cells.

Nodular lymphocytic lymphomas should generally be conceived of as systemic malignancies. This is in accordance with the concept that the small cleaved lymphocyte hematogenously migrates in a generalized manner to involve lymphoid tissue, including bone marrow, spleen, and lymph nodes. Small cleaved cells should therefore be thought of as migratory, in contrast to large cells, which tend to replicate and expand focally.

Diffuse Lymphomas

Diffuse, well-differentiated lymphocytic lymphoma (DWDL) comprises about 5% of all non-Hodgkin's lymphoma. If a leukemic phase is present, the illness is classified as chronic lymphocytic leukemia; DWDL represents an indolent illness with a favorable prognosis. Approximately 15% of patients with DWDL have a monoclonal immunoglobulin present in the serum, most often of the IgM kappa type.

Two variants of diffuse, poorly differentiated lymphocytic lymphomas (DPDL) are found in adults. The first, also known as diffuse, small cleaved cell lymphocytic lymphoma, affects adults over 40 years of age, confers a poor prognosis, and is generally composed of B malignant lymphocytes. The second variety, known as lymphoblastic lymphoma, characteristically affects young adults during the second and third decade of life. Lymphoblastic lymphomas are usually composed of lymphocytes with convoluted nuclei and are of T cell origin. The enzyme terminal deoxynucleo-

tidyl transferase is universally present. Mediastinal lymphadenopathy is common. Following initial responses to radiation or intense chemotherapeutic programs, there is a high frequency of relapse, particularly in the bone marrow and meninges.

Diffuse histiocytic lymphoma (DHL), also called diffuse large cell lymphoma, is the most frequently encountered diffuse lymphoma. This variety of lymphoma is composed of transformed lymphoid cells, most commonly of the B cell type, but rarely showing T cell characteristics. However, 40% of such DHL are of the null cell variety. DHL involves extranodal sites more frequently than any other type of lymphoma. Patients generally present with rapidly enlarging but localized tumor masses. Only 10%, however, involve the bone marrow at presentation. Bone marrow involvement, when it occurs, indicates a greater likelihood for central nervous system involvement. Organ infiltration and invasion characteristically develop as the disease evolves.

Immunoblastic lymphoma is a newly recognized category that had been formerly included in the DHL category by Rappaport. Immunoblastic lymphomas may be composed of either B or T cells, but the B cell types generally have plasmacytoid morphology. Immunoblastic lymphomas are those most often to develop in a setting of abnormal immunoregulatory environment, e.g., as occurs following renal allograft transplantation or autoimmune disease. Immunoblastic lymphomas may also develop in patients with previously diagnosed chronic lymphocytic leukemia, Waldenstrom's macroglobulinemia, or plasma cell myeloma. Such a transformation of a well-differentiated lymphoplasmactoid neoplasm to a more poorly differentiated aggressive lymphoma, usually of the B cell variety, is known as a Richter's transformation.[15a]

A particularly unusual form of immunoblastic lymphoma occurs in people from the Mediterranean region, particularly Sephardic Jews in Israel and North Africans. Extensive submucosal gastrointestinal infiltration occurs, resulting in a clinical malabsorption syndrome. Such immunoblastic lymphomas almost always secrete alpha heavy chains into the serum. Immunoblastic lymphoma has also been reported to develop in patients who have had a well-established diagnosis of angioimmunoblastic lymphadenopathy.

Refinements in the immunologic characterization of non-Hodgkin's lymphoma are currently in progress. For instance, monoclonal antibodies demonstrate a considerable heterogenity among the T cell lymphoblastic lymphomas. Most show phenotypes of stage II or stage III thymocytes. In contrast, cells from patients with T cell acute lymphoblastic leukemia generally have the stage I thymocyte phenotype. Furthermore, T cells derived from lymphoblastic lymphoma tend to show the common acute lymphoblastic leukemia antigen four times more frequently as the cells from T cell acute lymphoblastic leukemia.[42]

Nodular lymphomas generally represent noeplastic proliferations of lymph node follicular center B lymphocytes. Such cells tend to be migratory and present in a leukemic phase or in the bone marrow when light chain typing is performed. Unlike chronic lymphocytic leukemia cells, they generally have a low percentage of mouse rosette formation.

The new variety of T cell lymphoma recently described in Japan has been mentioned above. Paradoxically, the cells express the helper-associated antigen OK-T4 but appear to suppress B cell immunoglobulin secretion.[53]

Staging

Owing to the variable patterns of disease progression among the different histopathologic types of non-Hodgkin's lymphoma, a rigid, routine staging plan cannot be recommended for all patients. Although the Ann Arbor staging classification originally devised for Hodgkin's disease (see Chapter 1, Table 1–1) has been generally applied to the non-Hodgkin's lymphomas, it remains unclear whether the various stages confer prognostic significance for all pathologic varieties. The distinction between stage III and stage IV disease is relatively unimportant for practical purposes, since total nodal irradiation with curative intent cannot be applied to stage III patients, in contrast to Hodgkin's disease. This derives from the high likelihood of either hepatic and/or mesenteric nodal involvement in those patients with retroperitoneal lymph node involvement.[55] For the DHL, factors such as the degree of tumor bulk and particular organ involvement, such as the central nervous system or bone marrow, may be of more prognostic importance than the distinction between stage III

or IV disease. Nevertheless, staging is important, particularly if the therapeutic strategy is to deliver radiotherapy alone to stage I patients.

Non-Hodgkin's lymphomas are usually generalized in distribution. Of 170 patients at the National Cancer Institute who underwent thorough surgical staging, including exploratory laparotomy, only 6% with nodular lymphomas, and 30% with DHL were pathologically in the stages I and II.[7]

The peripheral blood should be examined carefully for lymphocytes with cleft nuclei, particularly in patients with poorly differentiated lymphocytic lymphomas. A chest x-ray should be obtained to evaluate the hilar and mediastinal areas. Bone scans are more sensitive in detecting bone involvement than routine skeletal x-rays. Either a lymphangiogram or a computerized axial tomographic scan of the abdomen and pelvis should be obtained. The lymphangiogram has the advantage of detecting retroperitoneal lymph nodes which are replaced but not enlarged. In contrast, the computerized axial tomographic scan holds the relative advantage of being able to detect splenic hilar, porta hepatic, and mesenteric lymph node enlargement, in addition to visualizing the retroperitoneal, para-aortic, and iliac nodes. A positive lymphangiogram confers an 80% likelihood of having either hepatic and/or mesenteric nodal involvement present outside the confines of the standard inverted Y irradiation portal.[8] Gallium scans are more sensitive in detecting lymph nodes involved by large cell lymphomas than well- or poorly differentiated lymphocytic lymphomas.[51] Bone marrow involvement varies according to the histopathologic type of lymphoma. Whereas 85% of patients with NPDL have bone marrow involvement, only 10% of DHL involve the bone marrow. A bilateral posterior iliac crest bone marrow biopsy provides the best yield in detecting bone marrow involvement. Laparoscopically directed liver biopsies confer an approximately 30% greater yield of detecting liver involvement than percutaneous liver biopsy.[8]

Staging laparotomy has limited usefulness in non-Hodgkin's lymphoma. Most often, the generalized spread can be detected by less invasive procedures. Furthermore, distinguishing between stage III or IV disease does not usually alter the therapeutic strategy. Staging laparotomy may have a role in the management of those patients with clinical stage I DHL, if one is prepared to treat pathologic stage I patients with radiotherapy alone.

Therapeutic Agents

Therapeutic options include chemotherapy, radiotherapy, combined modality therapy, or no specific treatment. Although combined modality therapy may be shown to provide the best results for patients with localized diffuse lymphomas, it has a potential for both short- and long-term toxicity. Furthermore, combined modality therapy has not been proven to be of superior benefit to either modality alone in terms of survival advantage in non-Hodgkin's lymphoma. Therefore, the clinician often must choose between chemotherapy or radiotherapy as the initial strategy. DHL should be treated with curative intent, since they confer an unfavorable prognosis, and combination chemotherapy programs make cure a real possibility. In contrast, current available therapy for the more indolent, nodular lymphomas has not been shown to prolong survival. Routine aggressive treatment of nodular lymphomas cannot be justified. Accordingly, there has been a revival of interest in the expectant observation and nontreatment of nodular lymphoma.[40] Although patients with advanced nodular lymphomas can live for 5–10 years with little or no therapy, it remains unclear what proportion of these patients can be managed effectively with no initial treatment.

Radiotherapy

Radiotherapy with curative intent for localized non-Hodgkin's lymphoma plays a more limited role than for early stage Hodgkin's disease, since the non-Hodgkin's lymphomas are less often localized and the pattern of abdominal involvement is different than in Hodgkin's disease. The much more frequent involvement of mesenteric lymph nodes and the liver by the non-Hodgkin's lymphomas precludes the application of total nodal irradiation with curative intent, since such locations are outside the standard inverted Y irradiation portal.

Nevertheless, involved-field radiotherapy can be curative when administered in tumorcidal doses to stage I or II patients. The dosage commonly employed is 3000–4000 rads delivered over a 3–4 week interval. Although stage I patients com-

prise only 5%–10% of all patients with non-Hodgkin's lymphoma, involved-field radiotherapy can effect a prolonged disease-free survival for 5 years or more in more than one-half of such patients.[6] The results in stage II disease are poorer, particularly with DHL, where disease-free survival following involved-field radiotherapy alone is approximately 25% at 2 years.[48] Relapse usually occurs at sites outside the initial irradiation portal, indicating the high likelihood of occult, micrometastatic disease, undetected at presentation despite complete staging evaluation. Administering extended-field radiotherapy, as in Hodgkin's disease, does not improve results. The sterilizing dose of radiation for histiocytic lymphoma may be higher than for other histopathologic types. For histiocytic lymphoma, 4500–5000 rads should be administered.[23]

Radiotherapy constitutes the primary mode of treatment for localized extranodal lymphomas. Since histiocytic lymphomas are the most frequent pathologic variety comprising primary extranodal disease, complete remissions often require the application of 5000 rads. Primary histiocytic lymphoma of bone requires a higher dose of irradiation, on the order of 6000 rads for local sterilization.[36]

Total-body irradiation given at a dosage of 5–15 rads 3–5 times a week to a total dose ranging from 100 to 250 rads has been advocated for treatment of advanced nodular, non-Hodgkin's lymphoma. Such treatment for stage III and IV nodular lymphoma patients results in approximately 60% 5-year survival and 25% disease-free survival.[20] The results are similar to those achieved with chemotherapy. A controlled, prospective randomized trial at the National Cancer Institute comparing total-body irradiation with chemotherapy for the treatment of nodular and diffuse lymphocytic or mixed stage III and IV lymphomas has shown both therapies to be equivalent. Combining both therapies does not appear to offer additional benefit.[57]

Single-Agent Chemotherapy

The response rates of advanced non-Hodgkin's lymphoma to single-agent chemotherapy are shown in Table 2–7. Alkylating agents are superior to vinca alkyloids; vincristine is superior to vinblastine. Procarbazine has some activity against histiocytic lymphoma and, being lipophilic, crosses the blood–brain barrier, as high doses of methotrexate requiring citrovorum rescue. L-Asparaginase has considerable activity against lymphocytic lymphomas, particularly those of the T lymphocyte types. 2-Deoxycoformycin has specific activity against T cell lymphomas, since the enzyme which it inhibits, adenosine deaminase, is found only in T cells and not B cells.[3]

TABLE 2–7 Response Rates (%) to Single Agents Used to Treat Non-Hodgkin's Lymphoma

Drug	Histiocytic Lymphoma	Lymphocytic Lymphoma
Nitrogen mustard	18	60
Cyclophasphamide	57	67
Vincristine	65	38
Vinblastine	28	19
Procarbazine	27	—
Doxorubicin	49	35
Bleomycin	40	44
BCNU	33	20
Dicarbazine	—	27
VP-16-213	36	58
VM-26	19	28
Methotrexate (high dose)	70	—
L-Asparaginase	—	60

Adapted from Bender RA, DeVita VT: Non-Hodgkins lymphoma. In Staquet MJ (ed): Randomized Clinical Trials in Cancer: A Critical Review by Sites. New York, Raven Press, 1978, pp 77–102.

Single-agent chemotherapy has a role in the treatment of the most indolent varieties of non-Hodgkin's lymphoma. DWDL confers an average survival in excess of 6 years, and it is best treated by the sequential use of single chemotherapeutic agents, usually commencing with an oral alkylating agent, sometimes in conjunction with intermittent corticosteroid administration. Such therapy, however, has not been shown to prolong survival.

NPDL is the most common indolent variety of non-Hodgkin's lymphoma and confers an average survival of 6 years. In a randomized clinical trial comparing the single oral alkylating agent, cyclophosphamide, with the combination of cyclophosphamide, vincristine, and prednisone (CVP), followed by total–lymph node irradiation, each resulted in equivalent 5-year survival rates.[17] Although single-agent maintenance chemotherapy prolongs remission for patients with indolent nodular lymphomas, it has been argued that prolonged alkylating agent therapy as maintenance treatment serves to hasten the development of drug resistance and reduces the subsequent remission rate with the same agent.

Combination Chemotherapy

The same principles underlying the development of effective combined chemotherapeutic programs for the treatment of advanced Hodgkin's disease have been applied to design therapies for non-Hodgkin's lymphoma. These principles include nonoverlapping toxicities, lack of cross-resistance among the agents employed, and intermittent administration to permit bone marrow recovery between cycles. Some combination programs employ a particular drug for a specific reason, such as its ability to penetrate the blood–brain barrier. Other programs employ specific drugs to capitalize on theoretical drug interactions, such as the COMLA[49] program, in which high-dose methotrexate has been shown to aid the penetration of cytosine arabinoside into the malignant cell. For the treatment of Hodgkin's disease, the optimal number of drugs in a given program appears to be four. For the non-Hodgkin's lymphomas, the optimal number, if any, remains undetermined. Many of the combination programs currently in use for the treatment of non-Hodgkin's lymphomas are described below in reference to specific therapy for the various histologic varieties.

Management

Advanced Nodular Lymphomas

With the exception of nodular histiocytic lymphoma, the nodular lymphomas tend to be indolent and naturally wax and wane with time. NPDL is the most frequently encountered type of nodular lymphoma, with an average survival of greater than 5 years. Interestingly, survival is not adversely affected by bone marrow or liver involvement in the nodular lymphomas. Following radiotherapy for apparent localized disease, or chemotherapy for advanced disease, relapse rates are constant and continuous up to 15%/year, even beyond 5 years. Thus, despite the term "favorable-prognosis" lymphoma applied to NPDL, patients ultimately die of their disease. Therapeutic programs for the treatment of nodular lymphomas are shown in Table 2–8. Response rates for combination chemotherapeutic programs are in excess of 90%, with complete remission rates averaging approximately 50%. Although approximately 75% of patients are alive at 5 years, only approximately 20% are disease free. Whereas achieving a complete remission may not be significant in terms of overall survival for NPDL, this may not be the case for nodular mixed lymphoma; for the latter, two studies have demonstrated that the survival of patients achieving a complete remission is greater than that of partial remitters.[1,12] Because prolongation of survival for patients with NPDL has not been demonstrated, some workers have advocated an initial no-treatment approach. Since nodular lymphomas eventually become aggressive and transform into diffuse lymphomas following a prolonged indolent course, it remains to be seen whether or not early or late treatment during the indolent phase has any effect upon this metamorphosis.

Palliative radiotherapy is a useful adjunct to chemotherapy in situations in which localized tumor bulk causes symptoms or organ dysfunction, such as in ureteral obstruction. As mentioned

TABLE 2–8 Therapeutic Programs for Patients with Stages III and IV (Advanced) Nodular Lymphoma

Program[a]	Author	Complete Response (%)	5-Year Survival (%)
Cyclophosphamide or chlorambucil	Jones et al.[22]	31–48	—
CVP (NCI)	Anderson et al.[1a]	67	75
COP	Luce et al.[29]	49	55
CHOP	McKelvey et al.[31]	69	—
MABOP	Bonadonna et al.[4]	53	—
Total-body irradiation	Johnson[19]	90[b]	68

[a] CVP and COP, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; MABOP,
[b] Overall response.

above, total-body irradiation is a reasonable therapeutic option, providing equal benefit compared with CVP in terms of remission induction and relapse-free survival, but with perhaps greater long-term toxicity resulting in variants of acute leukemia.[19]

In general, clinical trials have shown that, although complete remission rates are higher with CVP than with single alkylating agent therapy, overall survival results are equivalent. Similarly, continuous single-agent drug maintenance therapy prolongs remission duration, but does not improve survival. A recent randomized study in Milan demonstrated no significant difference in either relapse-free or overall survival with the addition of chemotherapy (CVP) to radiotherapy alone in patients with localized NPDL.[5]

Nodular mixed and nodular histiocytic lymphomas are relatively uncommon; they are thought to develop as NPDL dedifferentiates. Combination chemotherapy can achieve a complete remission for approximately 75% of patients with nodular mixed lymphoma. Unlike NPDL, the remissions appear to be durable. Nodular histiocytic lymphoma comprises only approximately 3%–4% of all non-Hodgkin's lymphoma. Similar to nodular mixed lymphoma, however, complete remissions, when achieved, remain prolonged. Thus, both nodular mixed and nodular histiocytic lymphomas are potentially curable and, accordingly, treatment with intense chemotherapeutic programs is justified.[12]

Diffuse Histiocytic (Large Cell) Lymphoma

Approximately 30% of patients with DHL present with pathologic stage I or II disease. At the University of Chicago, treatment with radiotherapy alone resulted in a median survival of 58 months for pathologic stage I patients, but only 18 months for pathologic stage II patients.[47] Similar results have been reported from the National Cancer Institute: pathologic stage II patients with DHL who received involved-field radiotherapy survived a median of only 33 months. Thus, radiotherapy alone provides adequate treatment for stage I patients, but not for stage II patients. Accordingly, stage II patients would be best treated with either chemotherapy alone or combined modality therapy. Either of these strategies would preclude the need for staging laparotomy. It remains to be demonstrated whether or not chemotherapy alone with combined modality therapy could improve results over radiotherapy for stage I DHL. Clinical trials are currently in progress to answer this question.[47]

As stated above, patients with advanced NPDL are at a continuous risk for relapse irrespective of whether or not a complete remission is achieved. This is not true for advanced DHL. Almost all patients with advanced DHL who either do not respond to initial chemotherapeutic programs or have only a partial response die of the disease within 2 years. Complete remission, on the other hand, confers a 40% chance of prolonged, unmaintained, disease-free survival for longer than 5 years.[35] It is therefore justifiable to describe current intense combination chemotherapeutic programs as potentially curative of advanced DHL.

Some of the combination chemotherapeutic programs administered to previously untreated patients with advanced DHL are listed in Table 2–9. Since 1970, complete remission rates have increased from 40% to greater than 70% as more intense treatment programs have been developed. Furthermore, approximately 70% of those achieving complete remission remain disease free for longer than 2 years, without maintenance treatment. Such treatment programs are myelosuppressive, and some contain a component designed to treat occult central nervous system disease.

With the cure of DHL becoming a reality, attention is now being focused upon identifying initial prognostic variables, such as histologic subsets and clinical characteristics to predict better a complete response. Advanced stage (III or IV), the presence of fever or weight loss, gastrointestinal masses greater than 10 cm in diameter, liver involvement, bone marrow involvement, hemoglobin less than 12 g/dl, and a serum lactate dehydrogenase level greater than 250 units/ml all adversely affect the attainment of a complete remission.[15] Such factors tend to reflect tumor cell mass. Although the overall rate of central nervous system involvement by DHL, usually manifested as lymphomatous meningitis, occurs in approximately 8% of patients, bone marrow involvement and possibly testicular involvement predict a higher risk.

When a diagnosis of lymphomatous meningitis is established, recommended therapy includes whole-brain radiotherapy plus intrathecal chemotherapy. Methotrexate is usually given intrathecally because it is effective in leukemic meningitis. Whether or not methotrexate is best given by repeated lumbar punctures or via an Ommaya reservoir remains an unanswered question. Approximately 85% of patients receiving such combined

TABLE 2–9 Therapeutic Programs in Previously Untreated Patients with Advanced Diffuse Histiocytic Lymphoma

Program[a]	Author	Complete Response (%)	Median Survival (months)
CVP	Nissen et al.[38]	42	0
C-MOPP	DeVita et al.[10]	41	9
CHOP/HOP	McKelvey et al.[31]	68/66	23
BACOP (NCI)	Schein et al.[44]	48	>17
BACOP (Farber)	Skarin et al.[45]	56	9
COMLA	Sweet et al.[49]	55	>33
M-BACOD	Skarin et al.[46]	80	>21
ProMACE-MOPP	Fisher et al.[14]	63	>19
COP-BLAM	Lawrence et al.[27]	63	—
ACOMLA	Newcomer et al.[37]	64	—

[a] CVP, cyclophosphamide, vincristine, and prednisone;

modality central nervous system therapy have improvement or attain good control of disease manifestations.[58] Total eradication, however, is infrequent. Because there is a proclivity to involve the central nervous system, some of the newer combination programs contain a component designed to treat the central nervous system at initial diagnosis in the absence of demonstrable lymphomatous meningitis. Examples are the use of high-dose methotrexate in the M-BACOD[46] and ACOMLA[37] programs, and procarbazine in the COP-BLAM[27] program. Clinical trials are being designed to test whether initial complete responders, particularly those with bone marrow involvement, derive any benefit from additional prophylactic central nervous system treatment consisting of whole-brain radiotherapy and intrathecal chemotherapy.

There is no currently available effective salvage treatment for those patients who fail to achieve a complete response or relapse. An additional therapeutic question to be answered by future clinical trials is whether it is advisable to give maintenance treatment to those who have achieved a complete remission. These issues and questions have only come about due to the dramatic improvement of therapies that now offer patients a reasonable chance for cure.

Diffuse, Poorly Differentiated Lymphocytic Lymphoma

DPDL and DHL comprise the two largest categories of unfavorable-histology non-Hodgkin's lymphoma. Like DHL, DPDL shows a high early relapse rate during the first year following a complete remission, approximately 33%. Relapse becomes less likely during subsequent years but, unlike DHL, does not become insignificant following 2 years. Results of some of the therapeutic programs employed to treat DPDL are shown in Table 2–10. Single alkylating agents result in an approximately 20% complete remission rate for patients with DPDL. Less than 10% of patients

TABLE 2–10 Therapeutic Programs for Patients with Diffuse, Poorly Differentiated Lymphocytic Lymphoma

Program[a]	Author	Complete Response (%)	Median Survival (months)
COP	Luce et al.[29]	50	16 (70)[b]
COP	Bagley et al.[2]	57	35 (62)
CHOP	McKelvey et al.[31]	68	12 (75)
HOP	McKelvey et al.[31]	55	

[a] COP, cyclophosphamide, vincristine, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; HOP, doxorubicin, vincristine, and prednisone.
[b] Percentage is given in parentheses.

treated with single agents survive longer than 2 years. Combination chemotherapy provides better results. Unlike DHL, however, there does not appear to be a subcategory of patients who enjoy prolonged remission and disease-free status following a complete remission.

New and Experimental Therapies

Phase II trials of new chemotherapeutic agents are currently in progress. Agents that show promise for activity against non-Hodgkin's lymphoma include methyl GAG, vindesine, VM-26, and gallium nitrate.[26,50,56] Delivery of agents by continuous intravenous infusion may improve their therapeutic index and thus be incorporated into combination chemotherapy programs. Infusions of bleomycin and vinca alkyloids are now being tested in this manner. Interferon derived from human leukocytes has been shown to effect a partial response in previously untreated patients with NPDL.[9] Antithymocyte globulin has been reported to cause regression of a T cell lymphoma.[13]

As the techniques for raising monoclonal antibodies improve, and more specific immunologic identification of lymphomas becomes possible, monoclonal antibody therapy with unique specificity may revolutionize therapeutic strategies in the future. For example, Miller et al. infused lymphoma cells from a patient with nodular lymphoma with murine myeloma cells.[33] This hybridoma secreted the human IgM lambda found on the cell surface of the patient's lymphoma cells. This particular IgM lambda was then used to raise a monoclonal antibody with specificity against the idiotype unique to the IgM lambda. Following eight intravenous infusions of this monoclonal anti-idiotypic antibody, the patient, who had been refractory to conventional chemotherapy, achieved a complete remission that persisted for 9 months following therapy.[33]

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CHAPTER 3

Angioimmunoblastic Lymphadenopathy

A Clinical View

David J. Straus

In the mid-1970s three groups of pathologists described a lymphomalike disorder associated with systemic symptoms and immunologic abnormalities. Frizzera[18] et al. termed the disorder angioimmunoblastic lymphadenopathy (AILD), Lukes and Tindle[33] immunoblastic lymphadenopathy and Radaszkiewicz and Lennert[46] lymphogranulomatosis X. There are three histopathologic features of the lymph nodes in this disorder (Figure 3–1):

1. There is an infiltration of the lymph node with lymphocytes, plasma cells, and "immunoblasts" at times also associated with eosinophils and histiocytes. The immunoblasts are large lymphoid cells that resemble histologic sections of mitogen-stimulated transformed lymphocytes. The cytoplasm is amphophilic on hematoxylin and eosin staining, azurophilic with the Giemsa stain, and pyronophilic with methyl green pyronine.
2. There is a proliferation of small "arborizing" blood vessels.
3. There is a diffusely distributed pale acidophilic interstitial material which stains lightly with periodic acid–Schiff. There may be distortion of the normal lymph node architecture with cellular depletion and "different forms of burnt-out germinal centers."[18]

These initial reports stressed histopathology and only briefly described the clinical features. Following their publication, a series of papers appeared reporting a myriad of clinical manifestations and associations. In collaboration with Dr. Charles Bagley and his colleagues at the University of Washington in Seattle, Dr. Peter Schauer and I looked at our own experience at Memorial Sloan-Kettering Cancer Center in New York City and that of our colleagues in Seattle from a clinical rather than a histopathologic point of view.[50] In this chapter our own experience and that in the literature are brought up to date. Five new cases

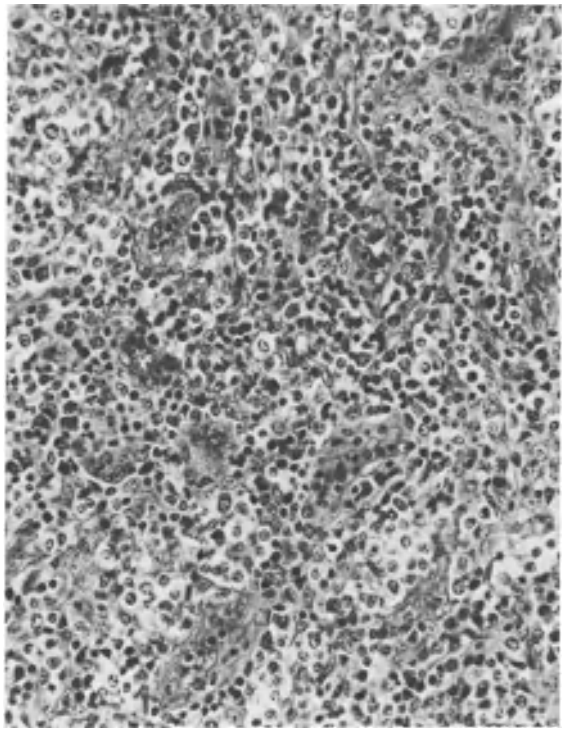

FIGURE 3–1 Photomicrograph of lymph node biopsy demonstrating angioimmunoblastic lymphadenopathy. Hematoxylin and eosin stain, X205. (From Daniel Filippa, M.D., Department of Pathology, Memorial Hospital, Sloan-Kettering Cancer Center, with permission.)

have been added to the 13 that we have previously reported.[50]

Clinical Features

As others have reported,[18,33] AILD is usually a disease of middle-aged or elderly individuals, although cases in younger individuals[27,42,44,65] and even in children[17,25] have also been described. The median age in our series was 59 years with a range between 45 and 78 years. There were 16 males and 2 females, although there does not seem to be a sex predominance in the literature. The clinical characteristics of these 18 patients are summarized in Table 3–1.

A common feature of cases in the literature has been a history of drug exposure, suggesting the possibility of an allergic etiology. The drugs implicated include dilantin,[19,33,37] penicillin,[19,33,46] griseofulvin,[33] cyclophosphamide,[46] chloroquine,[46] barbiturates,[35,37] sulfas,[33] procaine,[46] aspirin,[19] halothane,[19] liver extract,[52,53] dextroamphetamine,[55] methyldopa,[63] hydroxychloroquine,[51] pyrimethamine,[13] and insulin.[38] A 71-year-old shipyard worker developed AILD in association with asbestosis.[34] In our own series, only patient 17 had an association of the onset of symptoms with the administration of cephalexin for an upper respiratory infection.

Systemic symptoms were prominent in 14 of 18 patients, including weight loss in 11 patients, fever in 14, and night sweats in 11. All 18 patients had lymphadenopathy, and it was generalized in 16. The 2 patients with localized lymphadenopathy were also asymptomatic. Splenomegaly was present in 13 patients and hepatomegaly in 6. Four patients had significant ascites, and cytologic analysis of the ascitic fluid revealed atypical lymphocytes in 2 and reactive cells in the other 2. Cultures of the ascitic fluid were negative in all 4 patients, and 2 were treated empirically with isoniazid and ethambutol because of a positive tuberculin test.

Diffuse pulmonary infiltrates were seen on chest x-ray in three patients. Open lung biopsy was performed in one patient and showed an immunoblastic cell infiltration without evidence of infection. Four patients had pleural effusions; they were associated with ascites in three patients and with congestive heart failure in the fourth. Dyspnea and cough has been a feature of some patients in the literature with pulmonary involvement,[2,7,26,44,58,66] and some have developed severe respiratory insufficiency.[23,44,58] The radiographic findings include hilar and/or paratracheal lymphadenopathy and pleural effusion. Parenchymal lung involvement begins as diffuse interstitial nodular densities which may progress to a mixed alveolar and interstitial infiltrate.[32] The most extensive pulmonary function studies were reported by Zylak et al., who found a ventilation–perfusion imbalance associated with frequency dependence of compliance and a large closing volume in the presence of normal elastic recoil, probably indicating small airway disease.[66] As in one of our patients, lung biopsies showed infiltration of immunoblastic cells. Immunofluorescent stains with monospecific antisera of lung sections performed by Iseman et al. showed bright focal intracellular IgG and IgM and less intense staining for IgA in alveolar walls.[26]

A generalized rash was present in 8 of our 18 patients, and it was intensely pruritic in 3. The rash had a nonspecific appearance of a maculopapular erythema, although other features have been reported in the literature, including petechiae and purpura, erythroderma, and urticaria.[13,53,62] An exacerbation of the rash with drug exposure has been reported.[13,53] Skin biopsies have shown vasculitis in the capillaries of the dermis with perivascular collections of lymphocytes, histiocytes, and immunoblasts extending into the dermis.[19,53]

There are several disorders thought to have an autoimmune basis that have been associated with AILD, including Hashimoto's thyroiditis,[61] hypothyroidism,[19] uveitis,[14] Sjögren's syndrome,[37] pernicious anemia,[19] and Guillain-Barré syndrome.[27,28] The common association with autoimmune hemolytic anemia is described in the next section. Meningeal involvement,[41] even causing a spinal cord compression,[37] has been reported. One of our cases developed lung carcinoma, and an additional patient in the literature developed carcinoma of the pancreas.[11]

Laboratory Features

The laboratory features of our 18 patients are presented in Table 3–2. Anemia was present at time of diagnosis in 12 patients and was severe in 6. Of the 18 patients, 10 had a positive direct Coombs' test and a cold agglutinin was detected in 3. In all 3 patients the cold agglutinin was anti-I. Six patients (Nos. 4, 8, 10, 11, 15, and 16) had

TABLE 3–1 Clinical Features

Patient No.	Sex	Age	Weight Loss	Fever	Sweats	Clinical Stage	Splenomegaly	Hepatomegaly	Ascites	Pulmonary Involvement	Skin Rash
1	M	59	+	+	+	III	+	—	—	—	—
2	M	46	+	+	+	III	+	+	—	+	—
3	M	66	+	—	—	III	—	+	—	—	—
4	M	53	+	+	+	III	+	+	+	+	+
5	M	72	+	+	—	III	+	+	—	+	+
6	M	63	—	+	+	III	+	—	—	—	—
7	M	50	+	+	—	II	—	—	—	—	—
8	F	57	—	+	+	III	+	—	+	+	—
9	M	62	—	+	—	III	+	+	—	+	+
10	M	72	—	—	—	III	—	—	+	—	—
11	M	61	+	+	+	III	+	+	+	+	+
12	M	51	—	—	—	II	+	—	—	—	—
13	M	57	—	—	—	I	—	—	—	—	—
14	M	46	+	+	+	III	+	—	—	—	—
15	F	78	+	+	+	III	+	—	—	—	+
16	M	76	+	+	+	III	+	—	—	+	+
17	M	45	+	+	+	III	—	—	—	—	+
18	M	59	—	+	+	III	+	—	—	—	+

TABLE 3–2 Laboratory Features

Patient No.	Hematocrit (%)	WBC ($\times 10^3$/mm^3)	Eosinophils (%)	Platelets ($\times 10^3$/mm^3)	Reticulocyte count (%)	PB	BM	Coombs' Test	ANA	RF	Serum Immunoelectrophoresis	Anergy	Cryoglobulins	Cold Agglutinin
1	36	4.4	10	200	1.2	—	—	—	—	—	Low IgA (540), low IgA	—	ND	ND
2	41	7.8	10	192	1.0	—	—	+	ND	ND	Polyclonal increase	—	—	ND
3	35	7.3	2	260	ND	—	—	—	ND	ND	Absent IgA	—	—	—
4	16	11	0	48	1.2	+	+	+	ND	ND	Polyclonal increase	+	—	+
5	30	12	26	261	0.8	—	—	+	—	+	Absent IgA	+	ND	—
6	34	5.9	2	101	0.9	—	+	—	ND	ND	Polyclonal increase	+	ND	ND
7	39	13	29	287	1.4	+	—	+	—	—	Polyclonal increase	—	+	+
8	23	—	—	—	1.2	—	+	+	—	—	Decreased Ig's	—	ND	ND
9	30	5.5	0	179	1.0	—	+	+	ND	ND	Polyclonal increase	—	ND	ND
10	15	5.4	2	47	1.2	—	+	+	—	—	Polyclonal increase	—	—	ND
11	22	10	0	220	0.8	—	+	+	—	—	Polyclonal increase	ND	+	+
12	43	8.7	2	236	ND	—	—	—	—	ND	Normal	—	ND	—
13	47	12	0	282	1.7	—	ND	ND	ND	ND	Normal	ND	ND	ND
14	44	8.7	2	600	ND	—	—	—	—	+	Polyclonal increase IgG	ND	ND	—
15	20	5.7	11	35	1.8	—	—	+	+	ND	Polyclonal increase	ND	ND	—
16	18	8.2	26	281	3.9	—	+	+	ND	ND	Polyclonal increase IgG	ND	ND	—
17	36	22.2	9	158	ND	—	—	—	ND	ND	Monoclonal increase IgM kappa	ND	—	—
18	40	21.2	9	179	ND	—	+	—	ND	ND	Polyclonal increase IgG	ND	+	—

Abbreviations: WBC, white blood cell count; PB, peripheral blood plasmacytoid cells; BM, bone marrow plasmacytoid cells; ANA, antinuclear antibody; RF, rheumatoid factor; ND, not determined.

severe life-threatening autoimmune hemolytic anemia. In 4 it was due to a warm, Coombs' test–reactive antibody, while a cold agglutinin was responsible in the other two. The reticulocyte count was inappropriately low for the degree of hemolysis in all 6 patients, although the bone marrow aspiration smears showed erythroid hyperplasia in all 6. Anemia has been a feature of three-fourths of the cases reported in the literature, and red cell autoantibodies have been detected in one-half.[50] Six patients had a leukocytosis, a finding that occurred in 25% of cases in the literature.[50] Eight patients had eosinophilia, as compared to 31% in the literature,[50] and 2 patients had circulating plasmacytoid lymphocytes. Such cells and immunoblasts were also seen in the bone marrow in 8 patients, which was also described by Pangalis et al.[43] Three patients had platelet counts of less than 100,000/mm^3.

One patient had an antinuclear antibody with a nonspecific, diffuse staining pattern, and 2 had a serum rheumatoid factor in low titer. A patient has also been reported with a positive lupus erythematosus preparation with out a circulating anti-DNA antibody.[23] Circulating cryoglobulins were found in 3 patients. Schultz and Yunis reported a patient who developed AILD following exposure to liver extract associated with a serum mixed cryoglobulin containing IgG, IgA, IgM, and bound complement components.[52] This cryoglobulin formed a precipitin with liver extract which was found to reside in the IgA and IgM fractions.

Twelve patients had a polyclonal increase in serum immunoglobulins, and one patient (No. 17) had a low level of monoclonal protein (IgM kappa). Although a polyclonal increase in serum immunoglobulins has been reported in the literature in about three-fourths of the patients, monoclonal gammopathies have also been seen in a few.[12,18,28,45] Four patients had a decrease in serum immunoglobulins, which has also been previously reported,[62] and 2 had an absence of IgA.[9] Weisenburger et al. described two cases with decreased serum levels of total serum complement and C3 and C4 associated with pulmonary vasculitis.[64]

Defects in cell-mediated as well as humoral immunity have also been reported. Of 11 patients tested, 3 had anergy to recall skin test antigens, a finding that has also been reported in the literature.[6,15,29] Anergy has also been described with active skin sensitization with 2,4-dinitrochlorobenzene.[15]

Almost total unresponsiveness of peripheral blood lymphocytes has also been reported with several mitogens, antigens, and mixed lymphocyte culture.[6,8,21,29,45] In one patient the *in vitro* lymphocyte response to phytohemagglutinin and purified protein derivative was normal. The patient's lymphocytes responded to low concentrations of lipopolysaccharide, a B cell mitogen, while control lymphocytes failed to respond.[30] The percentage of circulating T lymphocytes has been low in some reports,[21,29,30,40,42] although in others[28,40] it has been normal. Bluming et al. demonstrated a lack of suppressor T lymphocyte activity in 2 patients with AILD with polyclonal hypergammaglobulinemia.[6] Garam et al. also described a decrease of natural killer lymphocyte activity in an AILD patient.[21] Kosmidis et al. found a decreased migration of lymphocytes to the skin following an inflammatory stimulus (Rebuck skin window technique) in another AILD patient.[29] This nonspecific abnormality has been found in patients with a variety of defects in cell-mediated immunity.

Hypercalcemia was reported in 2 patients with AILD.[20,60] One of these patients developed hypercalcemia in the setting of transformation of AILD into a lymphoma.[20] One of the patients with hypercalcemia was found to have a normal plasma parathyroid hormone level and no osteoclast-activating factor in cultured bone marrow cells. Bone marrow biopsy demonstrated immunoblasts adjacent to areas of active bone resorption.[60]

Outcome: Infection and Malignant Lymphoma

Only 5 of the 18 patients in our series are still alive with a follow-up period of between 3+ and 66+ months (Table 3–3). Only one of these patients, the longest survivor who had stage I disease, is completely disease free. The other 4 patients have achieved a partial response with treatment and have run a subacute course. The median survival for the entire group is 3 months. Eleven patients died of infections; these were bacterial infections in 9. Several of these patients had leukopenia secondary to cytotoxic chemotherapy, which may have contributed to their demise. Two patients died of opportunistic infections with *Pneumocystis carinii*. One patient died of a cerebral hemorrhage in the setting of chemotherapy-induced thrombo-

TABLE 3–3 Outcome

Patient No.	Diagnosis	Initial Treatment		Subsequent Treatment		Survival (months)	Cause of Death
		Therapy	Response	Therapy	Response		
1	8/76	RT	MR	Pred	NR	2	Sepsis
2	10/73	RT	MR	M-2 protocol	NR	11	Sepsis
3	5/77	Pred	NR	M-2 protocol	NR	2	Pneumocystis
4	3/74	CVP	NR	Splenectomy	NR	4	Pulmonary embolism, sepsis
5	9/76	Pred	NR	M-2 protocol	NR	1	Pneumocystis
6	3/79	Levamisole	NR	M-2 protocol	NR	1	CNS bleeding
7	10/79	Pred	NR	M-2 protocol	PR	4+	Alive
8	3/72	Splenectomy, Pred	MR	CVP, azathioprine	NR	9	Pseudomonas sepsis
9	3/76	Pred	MR	CTX, Pred	MR	3	Sepsis
10	5/76	Pred	NR	None		0.5	Sepsis, renal failure
11	2/74	CVP	CR	Pred, CVP, DXR, Proc	NR	21	Sepsis
12	5/77	COPP (1 cycle)		Pred	PR	30+	Alive
13	9/74	None		None		66+	Alive
14	11/81	CVP	PR	CVP	PR	3+	Alive
15	12/80	Pred	PR	Bleo, CVP	NR	2	Sepsis, pneumonia
16	3/81	Pred	NR	CVP	NR		
				Plasmapheresis	NR	5	Unknown
17	1/81	CTX, Pred	CR	Plasmapheresis	NR		
				CTX, Pred	PR	14+	Alive
18	1/80	CLB, Pred	NR	Misc. chemotherapy	MR		Sepsis,
				Interferon	NR	13	immunoblastic sarcoma

Abbreviations: Bleo, bleomycin; CLB, chlorambucil; COPP, cyclophosphamide, vincristine, procarbazine, and prednisone; CR, complete response; CTX, cyclophosphamide; CVP, cyclophosphamide, vincristine, and prednisone; DXR, donorubicin; M-2 protocol, see text; MR, minor response; NR, no response; Pred, prednisone; Proc, procarbazine; RT, radiotherapy.

cytopenia. A pulmonary embolism in the setting of sepsis contributed to the death of one patient.

AILD has a fulminant course ending in fatal infection in most patients. These infections seem to develop in the setting of the underlying immunosuppression caused by the disease and possibly exacerbated by prednisone and cytotoxic agents. Myelosuppression due to the latter treatments may have also contributed to the death of several patients. A minority of patients run a more subacute course, which may be helped by treatment, and an even smaller minority with localized disease seem to require little treatment. Claims of treatment success are, however, further confounded by reports of either temporary or durable spontaneous remissions.[17,19]

One of our patients developed a non-Hodgkin's lymphoma, which has also been reported in the literature.[3,16,25,33,36,39,57,65] Like most of the cases reported in the literature, this was an aggressive, rapidly growing large cell lymphoma (immunoblastic sarcoma). Lymphocyte surface marker studies of these lymphomas have been equivocal,[16,36,39] although some cases had prominent plasmacytoid features.[16,39] One patient developed a lymphoma with the morphologic features of a Burkitt's lymphoma and the characteristic cytogenetic translocation from the long arm of chromosome 8 to the long arm of chromosome 14 (46XX,t[8q−:14q+]). Antibody to Epstein-Barr virus capsid antigen was minimally increased, and no antibody to Epstein-Barr early antigen was present.[36] Only one case of AILD evolving to Hodgkin's disease has been reported.[65] Nathwani et al. reported a 35% incidence of progression of AILD to immunoblastic sarcoma in 23 patients in whom follow-up lymph node biopsies or autopsies were performed.[39] They found a decrease in the level of serum immunoglobulins in 7 of 14 patients, with the development of hypogammaglobulinemia in 6 in association with the malignant transformation. They also described the progression of malignant process from "clusters" to "islands" of malignant cells and eventually to diffuse lymph node involvement on serial biopsies.

Speculations on Pathophysiology

The etiology of AILD is not known, but there are certain facts about the disease that might lead to clues about its pathophysiology. The first of these, as mentioned earlier, is the hyperfunction of the B lymphocyte system. Polyclonal hypergammaglobulinemia and autoimmune hemolytic anemia are its most consistent manifestations. The association of acute immune phenomena such Hashimoto's thyroiditis, Sjögren's syndrome, pernicious anemia, and Guillain-Barré syndrome are also noteworthy.

The second fact is the defect in T lymphocyte function found in these patients. The defects include skin test anergy, decreased *in vitro* proliferative response to mitogens, antigens, and the mixed lymphocyte culture reaction, decreased *in vivo* migratory ability in response to skin inflammation, and decreased natural killer cell and suppressor lymphocyte activity.

A third fact is the presence of abnormal lymphocytes in patients with AILD. Random aneuploidy and chromosomal abnormalities without the presence of a marker chromosome were detected in the peripheral blood of one patient[59] and in lymph node preparations of 2 others.[24] The characteristic chromosome marker translocation of Burkitt's lymphoma between chromosomes 8 and 21, as previously mentioned,[36] was seen in an additional patient with AILD who developed a Burkitt-like lymphoma. By analogy, random chromosomal abnormalities in nonlymphoid bone marrow precursors are well-known antecedents of both idiopathic preleukemia[5] and several congenital diseases, such as Fanconi's anemia and Bloom's syndrome, in which acute nonlymphocytic leukemia develops in a high percentage of patients.

The causes of these abnormalities are unknown, but it is possible that drug exposure might play some role. A second possible mechanism might be virus infection. Intracytoplasmic virus-like particles resembling type A RNA virus particles were found on electron microscopy in a few of the proliferating cells in the lymph node of an AILD patient.[54] Several cases have been reported in which rubella infection antedated the onset of AILD.[31,42] Using an immunoflorescent technique, Krueger et al. were able to demonstrate intracytoplasmic rubella antigen in lymphoid cells of 11 of 14 patients with AILD.[31]

As suggested by Gleichmann et al.,[22] the membranes of certain lymphoreticular cells might be altered by sensitizing drugs or viruses, causing an immune response primarily of T lymphocytes. Helper T cells may be stimulated more than suppressor T cells, causing an imbalance and favoring

B cell proliferation and antibody production. As stipulated in Salmon and Seligmann's,[48] "two-hit" hypothesis for B cell neoplasia, a second oncogenic event (viral infection) might favor the emergence of a malignant B cell clone and the development of a B cell neoplasma. Nathwani et al.[39] and Neiman et al.,[40] using immunohistologic techniques, identified polyclonal immunoglobulins in the cytoplasm of immunoblasts in AILD patients. In contrast, surface marker studies demonstrated E rosette formation in 10% and 90% of immunoblasts in the respective studies of Rudders and DeLellis[47] and Klajman et al.[28] These findings suggest that the proliferating cells in AILD may be T cells and raise the possibility that the malignant transformation might occur in these T cells. As mentioned above, surface marker studies in immunoblastic sarcoma arising in the setting of antecedent AILD have been inconclusive.

Treatment

The treatment administered to our patients is summarized in Table 3–3. Minor responses were seen following local radiotherapy in 2 patients. High-dose prednisone resulted in minor regressions of lymphadenopathy in 3 patients, a partial response in 2 patients, and no response in 6 patients. A single alkylating agent, cyclophosphamide, combined with prednisone was effective in partially controlling the disease in one patient and provided a minor response in another. In contrast, chlorambucil/prednisone and bleomycin were unsuccessful. Combination chemotherapy was also tried in 12 patients. Cyclophosphamide, vincristine, and prednisone (CVP) resulted in 1 complete response, 1 partial response, and 5 failures. The M-2 protocol,[10] a combination of cyclophosphamide, vincristine, BCNU, melphalan, and prednisone, gave 1 partial response, while 4 patients failed to respond. Thus, attempts to blunt the immune response—presumably responsible for the debilitating symptoms often seen in AILD patients—with high-dose prednisone and cytotoxic chemotherapy have met with only limited success. The additional immunosuppression and myelosuppression caused by these agents might have exacerbated the infectious complications in a number of these patients.

Other approaches for modifying the immune response were also tried. Azathioprine was unsuccessful in a single patient. Levamisole is a drug that improves cell-mediated immune function by stimulating T lymphocyte differentiation and restoring effector function (by stimulating T phagocytes and T cells, including T suppressor cells).[49,56] It has resulted in clinical improvement in some AILD patients,[1,4,15] although in others no clinical improvement was seen despite improved lymphocyte proliferative responses *in vitro*.[8] One patient in our series failed to respond to levamisole. Interferon, a biologic modifier, was unsuccessful in 1 patient in our series. Plasmapheresis was performed in 2 patients in an attempt to remove circulating humoral factors that might be responsible for symptoms, but did not lead to a clinical improvement.

A rational approach to the treatment of this disease awaits a further understanding of its pathophysiology. Until then, treatment must be tailored to the individual patient, utilizing a conservative approach with observation, prednisone, and cytotoxic chemotherapy; it must be remembered that treatment may increase the risk for serious infectious complications.

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CHAPTER 4

Acute and Chronic Lymphocytic Leukemia

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Acute Lymphoblastic Leukemia in the Adult

Acute lymphoblastic leukemia (ALL) in adults is a relatively rare disease, comprising approximately 20% of the acute leukemias in patients above 15 years of age.[16] ALL is more common in children, comprising nearly 90% of the acute leukemias in patients under age 15 (Table 4–1). Many of the advances in the understanding of ALL and in the clinical management of the illness have been made by studying the pediatric population. Nevertheless, the approach to the disease in adults is quite similar, and today the possibility of cure of ALL has been realized in both the pediatric and adult populations.

The clinical manifestations of ALL are protean and generally nonspecific. Diagnosis rests on examination of the peripheral blood smear and bone marrow aspirate and biopsy. Recent developments have yielded the ability to diagnose ALL with more certainty.

TABLE 4–1 Relative Frequency of Acute Lymphoblastic Leukemia (ALL) and Acute Myelogenous Leukemia (AML) by Age

Age	(%) ALL	(%) AML
0–10	92	8
11–20	80	20
21–30	30	70
31–40	40	60
41–50	20	80
51–60	10	90
61–70	12	88
>70	18	82

Adapted from Boggs et al.[16]

Etiology

The leukemic process is characterized by the proliferation of a lymphocyte precursor clone of cells with a growth advantage arrested at an immature stage of lymphocyte development.[5] The bone marrow replacement and organ infiltration by leukemic cells results in the clinical picture of anemia, thrombocytopenia, circulating lymphoblasts, lymphadenopathy, and hepatosplenomegaly. The etiology of this process is not known.

Viral

Poresz et al. recently reported the isolation of a retrovirus from a patient with a leukemic T cell disorder.[94] Furthermore, specific antibodies to this virus, human T cell leukemia virus, have been found in a cluster of Japanese patients with acute T cell leukemia. Elevated antibody levels were detected in five of six patients with T cell leukemia, compared with none of 81 control patients.[51] This appears to be the first human example relating the neoplastic process to a specific viral etiology.

Genetic

The role of genetics in the etiology of ALL is unclear. A fourfold increased incidence of leukemia has been noted in the siblings of children with acute leukemia.[124 p. 1481] Chromosome abnormalities have been observed in acute leukemia. Bloomfield et al. documented karyotypic variation in 85% of patients with ALL.[14] Most common were hyperdiploidy in 53% and pseudodiploidy

(translocation) in 30%. Hypodiploidy, commonly seen in acute myelogenous leukemia, was present in only 17% of the cases studied. The significance of these abnormalities remains unclear.

Environmental

Environmental factors have been implicated in causing the leukemic process. For example, an increased incidence of ALL has been seen in survivors of the Hiroshima and Nagasaki nuclear bombing.[124 p. 1478]

Diagnosis and Classification

The diagnosis of acute leukemia is rarely difficult. The clinical picture, associated with a hypercellular bone marrow with sheets of immature cells replacing the normal bone marrow architecture, is diagnostic (Figure 4–1). Nevertheless, the proper classification of the acute leukemias by cell type may be problematic. The correct diagnosis of lymphoid leukemia as opposed to myeloid leukemia is very important for proper treatment. Furthermore, assigning the correct morphologic or immunologic subtype to a patient with ALL may have prognostic as well as therapeutic importance.

A combination of morphologic features, histochemical properties, specific enzyme levels, and immunologic subtyping has allowed for the accurate diagnosis and classification of ALL, and has aided in the understanding of the heterogeneous nature of this disease.

Morphologic Classification

The lymphoblast is classically described as a cell with a regular, folded nucleus with one prominent nucleolus, scant, agranular, basophilic cytoplasm, and a high nuclear/cytoplasmic ratio. In contrast, the classic myeloblast is a less regularly shaped cell with many nucleoli, granules and Auer rods in the cytoplasm, and a low nuclear/cytoplasmic ratio.[88,116] However, because of the morphologic heterogeneity of the acute leukemias, Bennett and others proposed a new classification, known as the French–American–British (FAB) system.[5] The FAB classification attempted to permit clear dis-

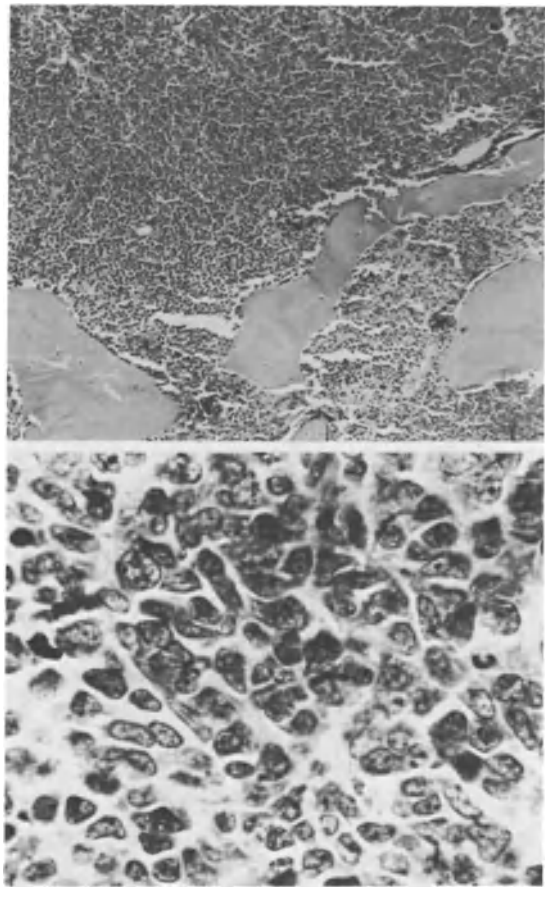

FIGURE 4–1 Morphology of acute lymphoblastic leukemia: bone marrow biopsy. **(a)** Low-power photomicrograph showing a bone marrow solidly packed with leukemic cells. There is total absence of fat and normal marrow elements. ×60. **(b)** High-power photomicrograph of same bone marrow. Primitive leukemic cells are obvious, with irregular nuclear membranes and finely dispersed nuclear chromatin. ×240.

TABLE 4–2 French–American–British Classification of ALL

Type	Cell Size	Nucleus	Nuclear Shape	Nucleoli	Cytoplasm	Basophilia	Vacuolization
L_1	Small	Homogenous	Regular	Indistinct	Scant	Slight	None
L_2	Large	Variable	Irregular	Prominent	Moderate	Moderate	None
L_3	Large	Stippled	Round	Prominent	Abundant	Intense	Prominent

Adapted from Bennet et al.[5]

tinction of myeloid from lymphoid leukemias and to classify subtypes within both groups in order to improve diagnostic ability and potentially identify variable features of the illness, in both presentation and prognosis.

The FAB classification of ALL into types L_1, L_2, and L_3 is described in Table 4–2 and illustrated in Figure 4–2. The L_1 cells are predominantly small cells, up to twice the diameter of the normal lymphocyte. The blast population is characteristically homogeneous. The L_2 cells are generally more than twice as large as the normal lymphocyte, more heterogeneous in size and shape, and exhibit a greater degree of cytoplasmic basophilia. L_3 blasts are termed Burkitt-like, owing to the abundant basophilic cytoplasm which displays marked vacuolization within the large, relatively homogeneous cell population.

The FAB group reviewed 100 cases of ALL and found 66 L_1, 29 L_2, and 5 L_3; 49 of the L_1 patients and 10 of the L_2 patients were in the pediatric age group, compared to 17 L_1 and 19 L_2 patients among the adult ALL cases. It is unclear whether survival differences between adult and pediatric ALL can be explained by the increased rate of L_2 cases within the adult population. It is observed that L_2 morphology within the pediatric population carries a poorer prognosis.[72] Differences between L_1 and L_2 morphology has not been possible with immunologic subtyping. However, the L_3 type has been found to be uniformly of the bone marrow–derived (B) lymphocyte type, and carries a poor prognosis.[6,45,72]

Histochemical Classification

In addition to morphology, the FAB system relies on cytochemical stains to divide the acute leukemias into lymphoid and myeloid types. It is recommended that all marrow aspirates be stained with either myeloperoxidase or Sudan black B, which are specific for the primary granules of the myeloblast.[5] These stains are always negative in ALL. This is crucial in proper diagnosis, as an occasional case with L_2 morphology will exhibit granules in the cytoplasm which will not take up either of the above stains.

Approximately 50% of all cases of ALL stain positively with periodic acid–Schiff stain.[72] This is not specific, as positivity is variable in the myeloid leukemias.[5]

Focal staining with acid phosphatase has been seen in a minority of cases of ALL. It has been found that these represent thymus-derived (T) cell leukemias, characterized by pleiomorphic blasts with folded nucleoli, a propensity for organ infiltration with mediastinal mass on presentation,

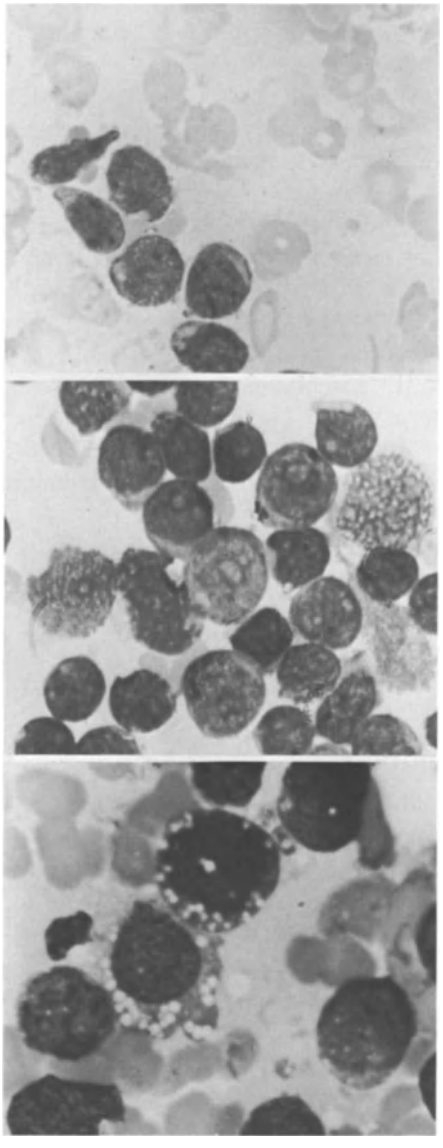

FIGURE 4–2 Morphology of acute lymphoblastic leukemia: bone marrow aspirate described by FAB classification. **(a)** L_1: predominantly small cells; homogeneous nuclear chromatin with regular nuclear shape; absent or indistinct nucleoli; scanty cytoplasm. ×600. **(b)** L_2: predominantly large cells but heterogeneity in cell size and in nuclear chromatin pattern; prominent nucleoli; variable cytoplasm, may be abundant. ×600. **(c)** L_3: Burkitt's type: cells large and homogeneous; dense nuclear chromatin with nucleoli; intensely basophilic; moderately abundant cytoplasm with vacuolization. ×600.

high initial blast counts, and high rates of central nervous system involvement.[72,118]

Enzyme Activity

Terminal deoxynucleotidyl transferase (Tdt) is an enzyme discovered nearly 20 years ago in the calf thymus extract.[80] The enzyme catalyzes the addition of deoxyribunucleotides to the end of a single-stranded series of bases without the need for a DNA template.[59] Elevated levels were first noted in calf thymocytes, and shortly thereafter they were found in a small number of normal human bone marrow cells.[22,59] In 1973, McCaffrey et al. reported a case in which elevated levels of Tdt activity were measured in the leukemic blasts of a child with ALL.[79] Subsequently, it has been determined that nearly all cases of ALL exhibit elevated Tdt activity in the leukemic blasts.[59,62,86]

Tdt positivity has been shown to predict response to therapy in patients with the blast phase of chronic myelogenous leukemia. Thirty patients with Philadelphia chromosome–positive chronic myelogenous leukemia in the blast phase were treated with vincristine and prednisone: 12 patients responded to this therapy, and 11 of the 12 had Tdt-positive blasts, despite clearly myeloid morphology in 5 of the 11.[77]

Tdt positivity is not specific for ALL. Approximately 5% of cases of acute myelogenous leukemia exhibit elevated Tdt levels. This may reflect abnormal gene expression within the blast population, or it may indicate that the target blast cell in these cases of acute myelogenous leukemia is pluripotent and exhibits features of both myeloid and lymphoid differentiation.[80]

The determination of Tdt activity in leukemic blasts may be performed by biochemical assay, radioimmunoassay, immunofluorescence, or histochemical techniques.[43,56,69,80] In light of the findings noted above, Tdt determinations should be performed on all patients with acute leukemia and chronic leukemia in the blast phase.

Varying levels of other enzymes, specifically adenosine deaminase and 5′-nucleotidase, have been observed in blasts of lymphoid origin.[67] The clinical utility of these enzyme levels has not been demonstrated.

Immunologic Classification

Immunologic subtyping of ALL has resulted from the explosion of knowledge over the past decade concerning the various components of the normal immunologic network. ALL has been divided into five subgroups based on the presence of the common ALL antigen (CALLA), the presence of the immune-related antigen (Ia), and the expression of normal cell surface markers of B cells and T cells.[83] These are summarized in Table 4–3 and Figure 4–3.

CALLA, a surface glycoprotein with a molecular weight of 98,000 daltons, is present on a majority of lymphoblasts.[9] It is also expressed on a small percentage of normal marrow cells that probably represent lymphoid precursor cells.[46,47,61,114]

Ia antigens are membrane glycoproteins, the expression of which is related to the HLA-D locus. Ia is expressed on a variety of cells, including B lymphocytes, monocytes, macrophages, a subpopulation of T lymphocytes, and some nonhematologic cells such as sperm and epidermal cells.[36]

The B cell is defined by the presence of surface membrane immunoglobulin. The pre–B cell is identified by the presence of intracytoplasmic heavy chain portions of IgM.[36]

The T cell is defined by the ability to form rosettes with sheep erythrocytes—which is observed in approximately one-half of all cases of T cell ALL—and reactivity with T cell–specific

TABLE 4–3 Immunologic Subgroups of ALL

Subgroup	CALLA	Ia	SmIg	T Antisera	Cμ
Common	+	+	−	−	−
Pre–B cell	+	+	−	−	+
T cell	−	−	−	+	−
B cell	−	+	+	−	−
Null cell	−	+/−	−	−	−

Adapted from Foon et al.[36]

Abbreviations: CALLA, common ALL antigen; Ia, immune-related antigen; SmIg, surface membrane immunoglobulin; Cμ, intracytoplasmic μ heavy chain.

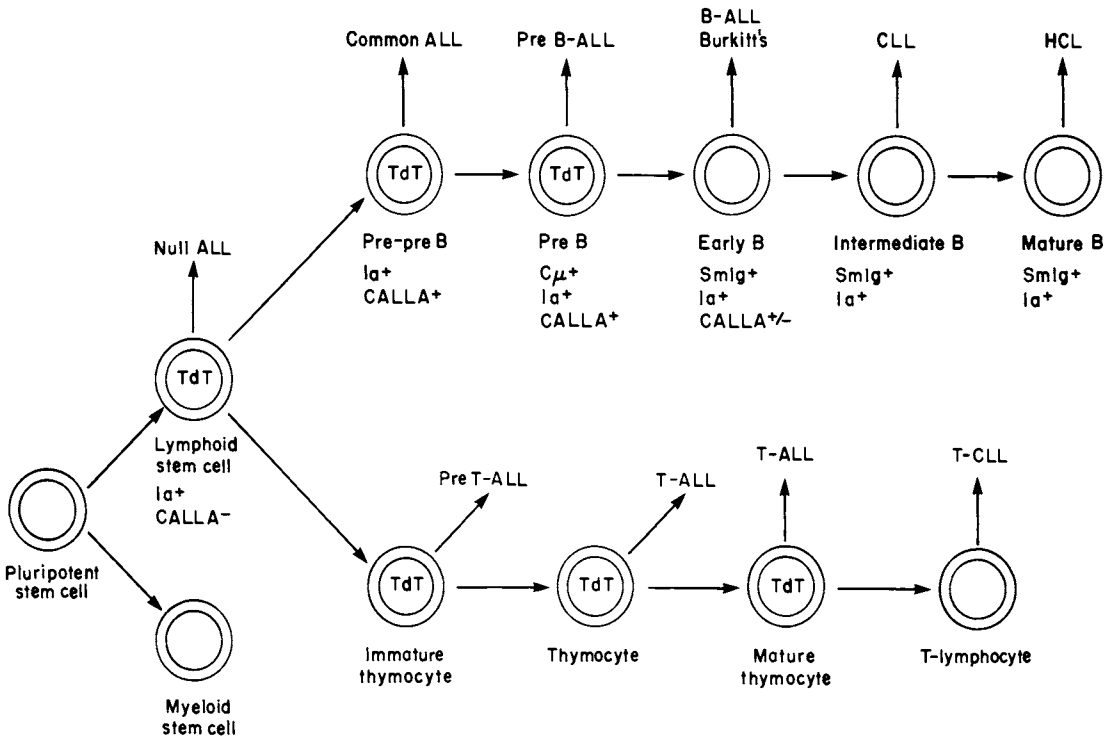

FIGURE 4–3 Lymphocyte development and stages at which maturation arrest will result in various immunologic subtypes of lymphoid leukemia. ALL, acute lymphoblastic leukemia; Cμ, intracytoplasmic μ heavy chain of IgM; CALLA, common ALL antigen; CLL, chronic lymphocytic leukemia; HCL, hairy cell leukemia; Ia, immune-related antigen; SmIg, surface membrane immunoglobulin; Tdt, terminal deoxynucleotidyl transferase. (Adapted from Foon et al.,[36] with permission.)

antisera.[34-36,98] The various immunologic subsets and incidence in the adult and pediatric population is shown in Table 4–4. At present, CALLA positivity appears to carry a good prognosis as an independent variable.[19] Both B cell and T cell leukemias do poorly when compared to the CALLA-positive group within the pediatric population.[18,19] However, it appears that the T cell type, as an independent variable, is not a negative prognostic factor. This is explained by the observation of high blast counts in patients presenting with T cell ALL. Greaves found that when patients are analyzed by white blood count at presentation, differences in prognosis between T cell and common ALL disappear.[45] It is hypothesized that adults with ALL have a poorer prognosis due to the higher incidence of the high-risk immunologic subtypes in null cell ALL.[36] Immunologic probing of leukemic cells will aid in the development of new therapies in adults with ALL, as well as help in the eventual unraveling of the process by which the leukemic cell population emerges.

TABLE 4–4 Relative Frequency of Immunologic Subgroups of ALL by Age

Subgroup	Children ≤ 15 years (%)	Adults (%)
Common	76	50.5
T cell	11.7	9.7
B cell	0.5	2.0
Null cell	12.7	37.8

Adapted from Greaves.[45]

Prognostic Factors

The poor prognostic features of childhood ALL have been clearly identified in very large series of controlled clinical trials by various institutions. High-risk factors include age greater than 10 or less than 2 years, male sex, initial white count in excess of 50,000/mm^3, the presence of adenopathy and/or hepatosplenomegaly, a mediastinal mass at presentation, central nervous system involvement at diagnosis, L_2 or L_3 morphology, B

or T cell markers, and the absence of CALLA on the leukemic cells.[84] In adults, the small number of patients and their uniformly poor performance status has not allowed clear identification of the high-risk group. However, certain clinical features, as well as immunologic and morphologic features, of adult ALL have been associated with poorer clinical performance.

Clinical Features

Sex, age, initial blast count, initial platelet count, and organ involvement are features that identify high-risk patients. Males do more poorly than females.[2,60] Patients over 40 years of age have a shorter mean survival time than those below 40.[108] Hepatosplenomegaly and/or lymphadenopathy at presentation has been associated with shorter remission duration, although the presence of isolated splenomegaly may indeed not be a high-risk factor.[38,76]

The white blood cell count at presentation is clearly most important in predicting remission duration. Blast counts in excess of 100,000 cell/mm^3 were a dire prognostic sign in many series.[2,54,76] Additionally, statistically significant longer remissions were demonstrated in patients presenting with an initial blast count of 10,000 cells/mm^3 or less.[76] Survival is also adversely affected by decreased platelet counts at presentation: survival was significantly worse in patients with presenting platelet counts below 50,000/mm^3.[108] Finally, the presence of central nervous system involvement at the time of diagnosis was associated with a significantly shorter survival.[76]

Morphologic and Immunologic Subtypes

The L_3 B cell ALL has a very poor prognosis in most series.[6,45,76] In rank order of increasingly good prognosis, the immunologic subtypes may be listed as follows: B cell ALL, T cell ALL, null cell ALL, and common ALL.[45] Whether these immunologic subtypes are independent prognostic variables or whether they reflect differences in clinical presentation remains unclear at the present time.

Treatment

Chemotherapy with cytotoxic agents remains the cornerstone of the treatment of adult ALL. The present-day approach has been developed as a result of multiple clinical trials in both pediatric and adult patients. The therapy is divided into remission induction (restoration of the normal hematologic state with less than 5% blasts in the bone marrow aspirate), early intensification once remission is obtained, maintanence therapy to maintain the leukemia-free state, and central nervous system prophylaxis or treatment.

Induction and Early Intensification

A comparison of various induction regimens is shown in Table 4–5. Initial complete remission rates with vincristine and prednisone in adults with ALL were approximately 40%–50%, and the re-

TABLE 4–5 Comparison of Various Remission Induction Regimens in Adult ALL

Author	Reduction Regimen	CR Rate (%)	Mean Duration of Remission (months)
Lister et al.[76]	V+P	40	
Jacquillat et al.[60]	V+P+DNR	73	11
Willemze et al.[122]	V+P+DNR	80	17
Gottlieb & Weinberg[44]	V+P+DNR	80	17
Gee et al.[40]	V+P+DNR	78	24
Lister et al.[76]	V+P+DNR+LASP	71	18.5
Gingrich et al.[41]	V+P+ARA–C	69	9.5
Ruggero et al.[108]	V+P+DNR+CY	78	22
Amadori et al.[2]	V+P+LASP	72	
Blalock et al.[12]	CY+P+DNR	89	36+

Abbreviations: V, vincristine; P, prednisone; DNR, daunorubicin; LASP, L-asparaginase; ARA-C, cytosine arabinoside; CY, cyclophosphamide.

missions were of short duration.[73,76] It was shown in a number of trials that the addition of daunorubicin or doxorubicin to the induction regimen increased the complete remission rate to 70%–80%.[60,86,122] The addition of L-asparaginase to the vincristine and prednisone combination yielded similar success.[2]

Cancer and Leukemia Group B (CALGB) showed that the addition of L-asparaginase after induction with vincristine and prednisone resulted in more long-term remissions than the simultaneous administration of the three drugs or than vincristine and prednisone used alone.[65] Lister et al., using vincristine, prednisone, daunorubicin, and L-asparaginase in combination reported a complete remission rate of 71%.[76]

The concept of early intensification has been sparked by the disappointing duration of remission in the adult population. Gee et al. reported a mean remission duration of 24 months with a program incorporating cytosine arabinoside, 6-thioguanine, L-asparaginase, and carmustine after induction with vincristine, prednisone, and daunorubicin.[40] Trials in the pediatric population by Aur et al.[3] and Muriel-Sackman et al.,[86,87] using cytosine arabinoside, cyclophosphamide, and methotrexate intensification, showed no improvement in remission duration.

It appears safe to conclude that the approach to adult ALL should include intensive induction with vincristine, prednisone, and an anthracycline, with the addition of L-asparaginase soon after. An example of such a regimen is shown in Table 4–6. The role of early and late intensification remains unclear.

Maintenance

The need for effective maintenance therapy in ALL is evidenced by the high relapse rates in patients in whom a complete marrow remission is obtained. Short remission durations were noted in studies using short intensive induction courses of chemotherapy.[21,66] Similarly, patients in complete remission who discontinue therapy relapse quickly. The most commonly used maintenance schedule has been methotrexate and 6-mercaptopurine.[125] Attempts to improve maintenance duration with the addition of intermittent vincristine, prednisone, cytosine arabinoside, and cyclophosphamide have not proved successful.[4,30,82,84] Sallan et al. reported improvement in duration of remissions with the addition of doxorubicin to vincristine, methotrexate, 6-mercaptopurine, and prednisone.[111] However, it is too early to know whether this improvement will stand the test of time. As there are so few patients remaining in complete remission, the question of duration of maintenance therapy remains unanswered in adults. In children, it appears that therapy can safely be stopped after 3 years of relapse-free maintenance therapy.[21]

Relapse

Hematologic relapse occurs in 70%–80% of adult patients with ALL in whom a complete remission

TABLE 4–6 An Accepted Treatment Schedule for Adult ALL

	Induction
Vincristine	2 mg i.v. days 1, 8, 15
Prednisone	40 mg/m² p.o. days 1–22 then taper
Daunorubicin	45 mg/m² i.v. days 1, 2, 3
L-Asparaginase	500 units/kg i.v. days 22–32
	Central nervous system prophylaxis
2,400 rads whole-brain irradiation after remission obtained	
Weekly intrathecal methotrexate, 10 mg/m² (maximum dose 15 mg) × 5 doses during central nervous system irradiation	
	Maintenance
6-Mercaptopurine	200 mg/m² p.o. × 5 days every other week
Methotrexate	7.5 mg/m² p.o. × 5 days every other week
Vincristine	2.0 mg i.v. weekly × 2 after every third course of 6-mercaptopurine and methotrexate
Prednisone	40 mg/m² × 14 days with vincristine, then taper
Omit 6-mercaptopurine and methotrexate during vincristine and prednisone treatment; maintenance doses should be adjusted according to peripheral counts	

Adapted from Gottleib and Weinberg V.[44]

is obtained.[23,40] Once in relapse, reinduction with vincristine, prednisone, anthracyclines, L-asparaginase, or cytosine arabinoside is successful in 80% of children and 50%–70% of adults.[68,126] These remissions are usually short, especially in patients having relapsed during maintenance chemotherapy.[126] New chemotherapeutic agents, such as m-AMSA, VP-16 and VM-26, are being used in refractory ALL with variable success.[78,102,103] High-dose therapy with either methotrexate or cytosine arabinoside is also being used.[20,127]

Central Nervous System Prophylaxis and Treatment

With improvement in the treatment of ALL in children, the incidence of central nervous system involvement by leukemic infiltration became significant, occurring in 50%–60% of patients in hematologic remission.[31] The problem of central nervous system involvement was realized as early as 1965, when Frei et al. of CALGB instituted intrathecal methotrexate in the induction regimen for childhood ALL.[37] This led to a series of trials over the years to examine the question of central nervous system prophylaxis. The St. Jude's group showed a decrease in incidence to less than 10% with the institution of whole-brain irradiation therapy and intrathecal methotrexate.[3] The same group showed similar results using 2400 rads craniospinal irradiation. This group and others have shown that the goal of central nervous system prophylaxis can be achieved with whole-brain irradiation therapy and intrathecal methotrexate, avoiding spinal irradiation, which causes bone marrow supression and a higher incidence of complications.[29,40,58,81] Attempts to avoid central nervous system disease by incorporating drugs that cross the blood–brain barrier have been ineffective.[68]

The problem of central nervous system leukemia was observed in the adult population as well, with an incidence of at least 40%–50%.[71] Omura et al. showed that 2400 rads whole-brain radiotherapy combined with five doses of intrathecal methotrexate in doses of 10 mg/m^2 (15 mg maximum dose) decreased central nervous system incidence from 11/34 in an untreated group to 3/28 in a treated group.[89] Unfortunately, no difference in the duration of hematologic remission nor survival could be seen between the two groups. The L_2 protocol series from Memorial Hospital, which incorporated intrathecal methotrexate without radiotherapy, had a 66% incidence of central nervous system disease in patients with white counts above 25,000/mm^3.[40] It seems that the combination of whole-brain radiotherapy and intrathecal methotrexate should be advocated at this time. Investigations into intraventricular drug delivery via Ommaya reservoir are currently under way.

In patients with documented central nervous system leukemia, treatment is approached by a combination of radiotherapy and intrathecal methotrexate or cytoxine arabinoside.[52] Clinical presentation of central nervous system leukemia is variable. Cranial nerve palsies are common due to leukemic infiltration of nerve sheaths at presentation. Diagnosis depends on cytologic examination of cytocentrifuge preparations of cerebrospinal fluid.[27] The CNS relapse rates are high in patients in whom intrathecal therapy is discontinued once central nervous system relapse has occurred.[48]

New Approaches to ALL

Despite advances in remission induction rates, the ability to cure ALL with conventional chemotherapy in the majority of adults remains elusive. New approaches have emerged to improve results in these patients.

Bone Marrow Transplantation

Bone marrow transplantation of 22 patients with HLA-compatible donors, aged 4–22 years, in second or subsequent remission, has resulted in long-term survival:[119] 50% remain in remission at 15–35 months after transplant. Only 18% of those transplanted in relapse have survived. Therefore, it appears that transplant of patients in second or subsequent remission is preferable to transplant once disease is refractory. When transplantation was compared with conventional chemotherapy in patients in second or subsequent remission, 11 of 24 of the transplanted group remained disease free at 17–55 months, whereas only 1 child of 21 treated with conventional chemotherapy remained in remission for more than 2 years.[63] Certainly, bone marrow transplantation is not available for all patients. However, if available, one should screen family members for HLA-compatible donors and should consider transplant for patients under 30 years old during second remission or possibly in first remission if they have "high-risk" disease (i.e., a high white count at presentation, and null, B, or T cell disease).[15]

Serotherapy and Specific Therapy

With the expansion of immunologic techniques and subtyping of ALL, passive serotherapy with anti-CALLA antibodies has been attempted in patients with CALLA-positive ALL. Initial responses have been noted; however, these have been very short lived.[101] The role of immunologic manipulation in the therapy of ALL remains experimental and speculative.[101] Finally, specific selective therapy has been reported with 2-deoxycoformycin, an inhibitor of adenosine deaminase in a patient with T cell ALL.[95]

Outlook

ALL remains a fatal disease in 70%–80% of affected adults. It has been seen that the favorable results in adult ALL have more or less been confined to adolescents and young adults, with the "older" population faring less well.

The challenges seem clear. The role of both early and late intensification requires further study. Furthermore, more precise designations of poor prognostic signs need to be made in the adult population so that more intensive treatment may be attempted. The immunologic approach to therapy is in its infancy and clearly needs development. It is hoped that these advances will come quickly and improvement in long-term survival will be realized.

Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) was described by Dameshek as "an accumulative disease of immunologically incompetent lymphocytes."[26] This concise definition describes the majority of patients with CLL, who present with physical findings and laboratory data that are consistent with Dameshek's theory. CLL is a disease of older persons: less than 10% of patients present before the age of 50 years. Men are about two times as likely to develop CLL as are women.[25] As in all forms of leukemia, CLL is more common in the white than in the black population.[24]

The diagnosis of CLL is based on the minimal requirement of an absolute and sustained lymphocytosis in the peripheral blood of no less than 15,000 cells/mm^3. The bone marrow is hypercellular and more than 40% of the cells are lymphocytes. The lymphocytes in peripheral blood and marrow are of the small, well-differentiated type (Figure 4–4). Slowly enlarging lymph nodes and gradual enlargement of the liver and spleen due to the accumulation of neoplastic lymphocytes may occur early or late in the course of the disease. The immunologic incompetence of the expanding lymphocyte population expresses itself in hypogammaglobulinemia with predisposition to infections, and in the emergence of such autoimmune phenomena as the production of antibodies against host red blood cells.

Biology

Lymphocytes in Chronic Lymphocytic Leukemia

CLL primarily represents a clonal proliferation of B lymphocytes (Figure 4–2). The malignant cells have immunoglobulin molecules on their surfaces bearing the same idiotype and light chain type. The most common surface immunoglobulins are IgM and IgD. The finding of two heavy chain classes does not preclude the concept of monoclonality, as the IgD and IgM have the same idiotype specificity and presumably reflect a stage of differentiation of normal B lymphocytes.[39,49] CLL lymphocytes also express the Ia antigen, the receptor for C′3, and the receptor for the Fc portion of immunoglobulin.[28,105,123]

Studies of the clonal origin of CLL have used glucose-6-phosphate dehydrogenase (G6PD) as a marker in patients who are heterozygous at this locus. Skin tissue from these patients manifested both A and B G6PD types. However, as one would predict, the CLL B lymphocytes manifested only one type of G6PD. In contrast to the neoplastic B lymphocytes, the T lymphocytes, granulocytes, erythrocytes, and platelets displayed both enzyme types in proportions similar to those found in skin. These findings indicate that CLL involves only committed B lymphocyte progenitors. This is in contrast to chronic myelgenous leukemia, in which similar studies have shown that a multipotent marrow stem cell is affected, with expression of one type of G6PD in granulocytes, erythrocytes, platelets, and some lymphocytes.[32]

About 1% of patients with CLL demonstrate predominantly T lymphocytes in their peripheral blood. These lymphocytes form rosettes with sheep red blood cells and do not bear surface immunoglobulin.[110] The T cell form of CLL appears to carry a poorer prognosis than the B cell type. Skin involvement is seen frequently in these

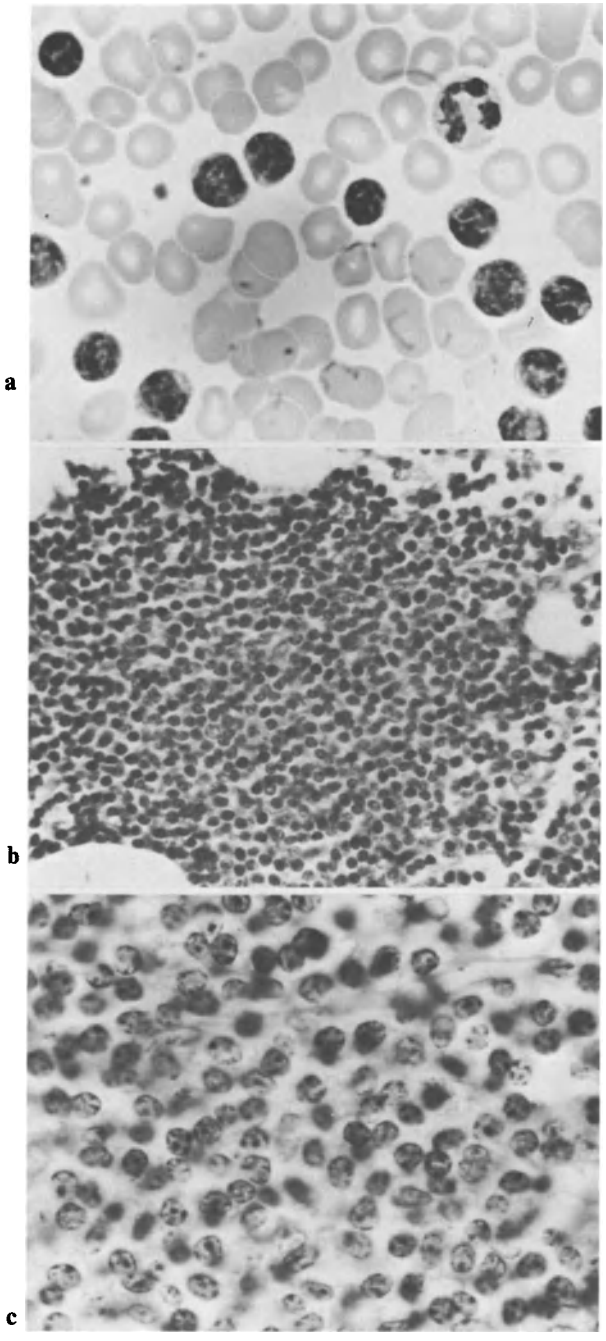

FIGURE 4–4 Morphology of chronic lymphocytic leukemia: peripheral blood and bone marrow. **(a)** Peripheral blood smear showing predominance of small, well-differentiated lymphocytes. ×750. **(b)** Bone marrow biopsy showing a nodular aggregate of neoplastic cells. The pattern may also be one of diffuse lymphocytic infiltration interspersed with normal marrow, or it may be one of solid replacement with sheets of neoplastic lymphocytes. ×150. **(c)** High-power photomicrograph of a bone marrow biopsy showing the uniform small size of the leukemic cells with the typical "checkerboard" chromatin pattern of the nuclei. ×320.

patients and standard forms of therapy for CLL are less effective.[120]

Etiology

The etiology of CLL is unknown. There are familial clusterings of CLL, but no firm genetic basis for the disease has been found.[13,50] Exposure to radiation or other potentially mutagenic agents such as alkylating agents does not appear to predispose to CLL. Consistent chromosomal abnormalities are not found. To date, there have been no convincing data to support a viral etiology for CLL.

Clinical Staging

Rai System

A major contribution to understanding the natural history of CLL is the clinical staging system devised by Rai et al.[96] In this system a patient is

TABLE 4–7 Rai Criteria for Clinical Staging of CLL at the Time of Diagnosis

Stage	Definition
0	Lymphocytosis alone Absolute lymphocytosis of 15,000/mm³ in peripheral blood 40% marrow lymphocytosis
I	Lymphocytosis and enlarged lymph nodes
II	Lymphocytosis and splenomegaly, hepatomegaly, or both
III	Lymphocytosis and anemia: hemoglobin <11 g/dl or hematocrit <33% Lymphadenopathy or hepatosplenomegaly not required Nature of anemia not specified
IV	Lymphocytosis and thrombocytopenia: platelet count <100,000/mm³ Anemia not required Lymphadenopathy or hepatosplenomegaly not required Nature of thrombocytopenia not specified

Adapted from Rai et al.[96]

assigned to a stage at the time of diagnosis. The prognosis for that patient is related to the stage of disease at presentation. The stages of the disease reflect Dameshek's concept of the "accumulative" nature of CLL. Stage 0 is lymphocytosis alone. Stages I and II reflect increasing tumor mass with lymphocytosis and lymphadenopathy (stage I) and/or hepatosplenomegaly (stage II). Anemia and thrombocytopenia, which characterize the more advanced stages III and IV, also primarily reflect tumor mass, with bone marrow replacement leading to decreased normal hematopoiesis and splenomegaly contributing to decreased red blood cell and platelet survival.[121] The staging system is outlined in more detail in Table 4–7.

The Rai staging system has been applied prospectively to a large series of patients. As seen in Table 4–8, the majority of patients are in stage I or II at the time of diagnosis. In the Rai et al. group of 125 patients studied both retrospectively and prospectively, 54.4% were in stage I or II;[96] in a prospective group of 196 patients reported by Binet et al., 57.6% were in stage I or II.[10]

The application of the Rai staging system has contributed to an improved understanding of the natural history of CLL by defining the effect of clinical stage on survival. The median duration of survival from the time of diagnosis of 125 patients studied by Rai et al.[96] is plotted in Figure 4–5. The overall median survival was 71 months for the entire group. However, the survival was dependant on the clinical stage. The patients in stage 0 had the best survival, with only 4 of 22 patients dead at 144 months. The median survival for stage I patients was 101 months; for stage II patients it was 71 months. Patients in stages III and IV had an identical median survival of 19

TABLE 4–8 Clinical Stage at Diagnosis of CLL

Stage	Rai et al.[96] (N = 125)	Binet et al.[10] (N = 196)
0	17.6	25.0
I	23.2	29.6
II	31.2	28.0
III	16.8	8.1
IV	11.2	9.6

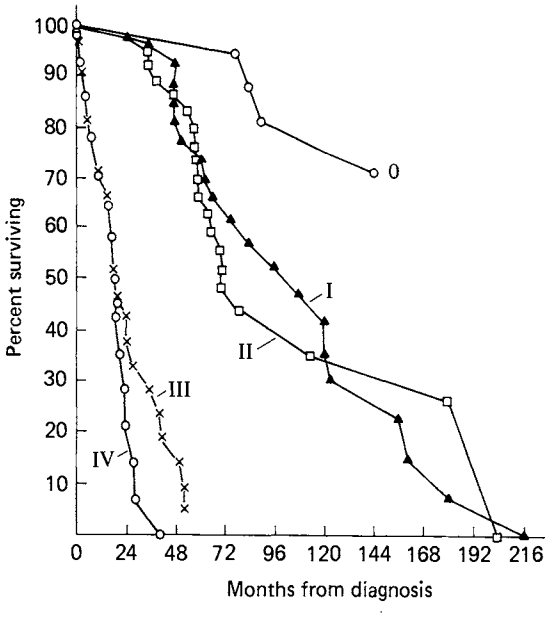

FIGURE 4–5 Median survival from time of diagnosis of 125 patients staged by the Rai criteria. Stage 0, N = 22 patients; I, N = 29; II, N = 39; III, N = 21; IV, N = 14. (Adapted from Rai et al.[96])

months. Survival according to sex was not different when adjusted for stage and patient age. Moreover, survival according to age at diagnosis was not an independent factor when adjusted for stage and sex.[96] When the Rai staging criteria were applied to two other large series of patients, the survival curves by stage at diagnosis were comparable to those shown in Figure 4–5.[17,53] Subsequently, other series of patients have been studied by these criteria and the validity of predicting survival in CLL by stage has been confirmed.[91,92,109]

International Workshop on CLL System

A new staging system was proposed recently by the International Workshop on CLL.[11] This system is basically a revision of the Rai system and has the advantage of using fewer clinical groups. In addition, the revised system does not consider isolated splenomegaly or hepatomegaly as a separate stage, but considers organ enlargement in a setting of all areas of lymphatic involvement. Clinical enlargement of the spleen, the liver, and lymph nodes in the cervical, axillary, and inguinal regions is considered. Each of these five anatomic regions is considered as one "area" of involvement. The International Workshop on CLL recommends that the revised classification be integrated with the Rai system. The revised system is shown in Table 4–9. When this system is integrated with the Rai system, a patient is staged according to Table 4–10. Combining both systems allows the natural history and response to therapy of the subsets to be more accurately studied.

The median survival of a large group of patients studied prospectively by Binet et al. using the revised system is shown in Figure 4–6.[10] At 40 months, the median survival of patients in stage A was 92%, stage B 62%, and stage C less than 20%. The revised system reconfirms the value of the clinical stage of the patient in predicting survival in CLL.

TABLE 4–9 Revised Classification Proposed by the International Workshop on CLL

Clinical Stage	Definition
A	No anemia or thrombocytopenia; less than three areas of lymphoid enlargement[a]
B	No anemia or thrombocytopenia; three or more areas of lymphoid enlargement
C	Anemia and/or thrombocytopenia; zero to five areas of lymphoid enlargement

From Binet et al.[11]

[a] The spleen, the liver, and the cervical, axillary, or inguinal nodes constitute the five areas of possible lymphoid enlargement.

TABLE 4–10 Staging of CLL Using Both the Rai System and the Revised System of the International Workshop on CLL

Revised System[a]	Rai System[b]	Patient Stage
A	0	A(0)
A	I	A(I)
A	II	A(II)
B	I	B(I)
B	II	B(II)
C	III	C(III)
C	IV	C(IV)

[a] See Table 4–9.

[b] See Table 4–7.

Correlation of Clinical Stage with Other Features

A major goal of the prognostic staging systems described above is to increase our understanding of the basic biology of CLL. It can be assumed that stages 0–II (Rai system, Table 4–7), or stages A–B (revised system, Table 4–9), represent early disease and stages III–IV, or stage C, represent late disease. Early in the disease, serum immunoglobulins may be normal but they decrease progressively with stage.[33] Bone marrow biopsy patterns appear to mirror the progression of disease.[75,106] Early in the disease the infiltration is interstitial, with some degree of replacement of normal hematopoietic tissue by mature lymphocytes. The pattern may progress with clinical stage to become nodular, and finally diffuse, with massive replacement of normal hematopoietic tissue as well as fat cells. Further investigation of this type of progression of immunologic and morphologic events will lead to increased insight into the etiology of CLL. Eventually, this may allow earlier therapeutic intervention to alter the course of the disease.

Immune Disorders

CLL is primarily a disorder of the B lymphocytes, the lymphocytes that govern the humoral immune response. It is not surprising, therefore, that CLL

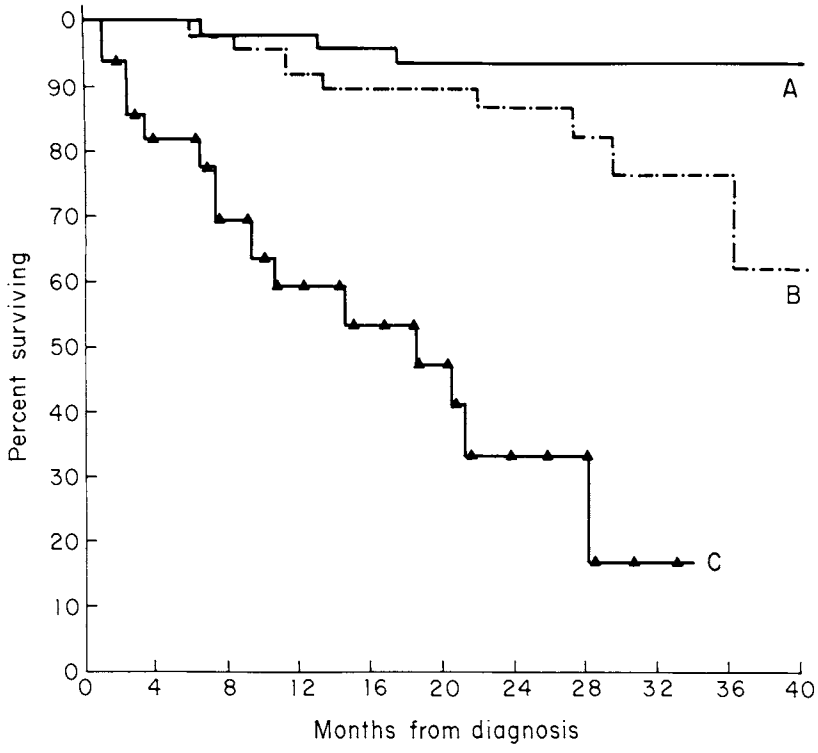

FIGURE 4–6 Median survival from time of diagnosis of 196 patients staged by criteria of International Workshop on CLL. Stage A, $N = 102$ patients; B, $N = 67$; C, $N = 27$. (Adapted from Binet et al.[10])

may be accompanied by disruption in the normal synthesis or function of immunoglobulins.

Autoimmune Hemolytic Anemia

Approximately 20%–30% of patients with CLL demonstrate autoantibodies against red blood cells.[70,93,104] In most of these patients, this results in minimal or no hemolysis, or a compensated hemolytic anemia. Most commonly, the antibody is of the IgG type and does not fix complement. In a minority of patients, the hemolytic anemia requires therapeutic intervention with corticosteroids and, less commonly, splenectomy. Treatment of the underlying disease with an alkylating agent such as chlorambucil may not result in remission of the hemolytic process. A unique approach using plasmapheresis and immunoabsorption with protein A has been described. Protein A is a staphylococcal cell wall constituent which binds the Fc portion of IgG. The patient reported demonstrated a rapid clinical recovery after this novel approach.[8]

Autoimmune Thrombocytopenic Purpura

Thrombocytopenia in CLL may be caused by three factors, alone or in combination. There may be decreased platelet production due to massive bone marrow replacement by neoplastic lymphocytes. Second, splenic enlargement with platelet sequestration may lead to decreased platelet survival. Third, autoantibodies directed against platelets may contribute to decreased platelet survival.[107] It is important to recognize the third "autoimmune" cause of thrombocytopenia, as the therapy and prognosis are different from thrombocytopenia due to infiltrative disease. For example, a patient assigned to stage IV (Rai system) because of thrombocytopenia due to immune destruction has a better prognosis than a patient in the same stage who has other causes for the thrombocytopenia. If a reliable test for antiplatelet antibodies is available, this may aid the clinician in differentiating the cause of the thrombocytopenia. Corticosteroids and/or splenectomy are the treatment of choice in this setting.

Disorders of Immunoglobulin Synthesis

Approximately 5% of patients with CLL demonstrate a monoclonal gammopathy by serum protein electrophoresis. The monoclonal peaks usually do not exceed 2 g/dl and may be either IgG or IgM. The monoclonal protein is rarely of clinical importance unless it is a cold agglutinin or, equally rarely, a cryoglobulin (1).

More commonly in CLL, hypogammaglobulinemia is found. Serum immunoglobulins may be normal early in the disease but fall to low levels

in the later stages. In one series of 48 cases, IgA was most frequently depressed (75% of all cases), IgM was depressed in 63% of the cases, and IgG was low in 17%.[33] The major clinical consequence of this acquired hypogammaglobulinemia is increased susceptibility to bacterial infection. There is no evidence to suggest that prophylactic gamma globulin is useful in preventing infection in this setting.

Therapy

Unlike the dynamic and exciting approach to the treatment of patients with ALL, the approach to treatment of patients with CLL has been, appropriately, an indolent one. Radiotherapy and chemotherapy are both active modalities in CLL treatment. The concept that treatment did improve symptoms but did not improve survival in CLL was introduced in 1924 by Minot and Isaacs.[85] Over 50 years later, there was little evidence to contradict this concept.[57,115]

A major contribution to more recent studies of response to therapy in CLL is the Rai staging system and the revised system of Binet (Tables 4–7 and 4–9). The concept that patients with stage 0–II disease have a much longer survival than patients with stage III–IV disease opens up a new arena for designing adequate protocols to study the effects of treatment on survival.[117]

Chemotherapy

Chemotherapy is the most common treatment for patients with CLL. Alkylating agents such as chlorambucil have long been the cornerstone of the chemotherapeutic approach. Lymphocytes are particularly susceptible to alkylating agents, and alkylating agents are well suited to a disease such as CLL because they are cell-cycle nonspecific. As such, they are toxic to the neoplastic lymphocytes without requiring these long-lived, slowly proliferating cells to be in a rapid growth phase. Additionally, alkylating agents are suitable for intermittent or "pulse" therapy for CLL. In such a program, normal marrow cells have an opportunity to recover from the toxic exposure long before the regeneration of the slowly proliferating neoplastic lymphocytes.

In 1971, CALGB instituted a randomized chemotherapy protocol to study patients with stage III and IV CLL.[113] The three protocol arms were high-dose intermittent chlorambucil and prednisone, low-dose continuous chlorambucil and prednisone, and prednisone alone. Corticosteroids may be therapeutic in CLL for two reasons: First, they may cause rapid regression of lymphoid tissue.[90] Furthermore, patients with stage III and IV disease may have an autoimmune component to the anemia or thrombocytopenia and corticosteroids may be beneficial in this setting.

Ninety-six patients were studied in the CALGB study. The "prednisone alone" arm was discontinued when 75% of the patients failed therapy within 7 months. Failure was described as any one of the following conditions: (a) no evident benefit from therapy without progression of disease after 4 months; (b) appearance of localized disease requiring radiotherapy; (c) relapsing disease unresponsive to increased drug dose; or (d) drug toxicity that precluded further drug usage.

The two chlorambucil arms were then compared by a number of parameters. As can be seen from the results presented in Table 4–11, there was no significant difference in remission induction or in median survival between the two chlorambucil-based arms. In addition, the hematologic, gastrointestinal, and infectious complications were not significantly difference between the two arms. Patients who responded to either therapy were the ones who eventually became the longer survivors of the disease.

Our present recommendation for patients with stage III or IV CLL is that they should be treated with chemotherapy. We favor intermittent chlorambucil. In elderly patients, 6 weeks of prednisone "induction" may be poorly tolerated, so we recommend individualization of the corticosteroid regimen. There has been no satisfactory program developed for patients who become resistant to standard alkylating agent therapy, although programs of combined chemotherapy have been used with some success.[74,92]

There is no standard approach to the treatment of patients with stage 0–II disease. In the absence of convincing evidence that treatment prolongs survival in this group of patients, we recommend treating these patients conservatively for control of symptoms with local radiotherapy or with short courses of chlorambucil alone or with corticosteroids.

Radiotherapy

Radiotherapy has been a mainstay of treatment for CLL since it was first reported in 1924.[85]

TABLE 4–11 Results of CALGB Randomized Protocol Comparing Intermittent Versus Daily Chlorambucil and Prednisone in Stage III–IV CLL

Results	Daily Chlorambucil[a]	Intermittent Chlorambucil[b]
Complete remission (%)	12.8	8.9
Partial remission (%)	25.6	39
Complete + partial remission (%)	38	47
Median time to failure (months)	7	16[c]
Median survival from onset of protocol (months)	21	37[c]

Adapted from Sawitsky et al.[113]
[a] Chlorambucil, 0.08 mg/kg p.o. daily with prednisone induction (0.8 mg/kg/day × 2 weeks, 0.4 mg/kg/day × 2 weeks, 0.2 mg/kg/day × 2 weeks) followed by prednisone maintenance, 0.5 mg/kg/day × 7 days every 28 days. Chlorambucil dose modified by 0.02 mg/kg/day increments as needed.
[b] Chlorambucil, 0.4 mg/kg p.o. every 28 days with prednisone induction and maintenance as above. Chlorambucil dose modified by 0.2 mg/kg/dose as needed.
[c] Difference not significant.

Splenic irradiation is the most common method employed. This not only results in shrinkage of the spleen, but also allows for radiation to large quantities of lymphocytes in the blood as it perfuses the spleen. Controlled studies comparing splenic radiotherapy and chemotherapy in stage-matched patients have not been performed. Palliative radiotherapy to areas of symptomatic disease (e.g., mediastinal nodes causing bronchial obstruction, disfiguring cervical nodes, etc.) can be extremely useful during the course of the disease.

Mediastinal (or thymic) radiotherapy as primary treatment for patients with CLL was originally reported as yielding good results with only mild toxicity.[99] However, a similar study undertaken by CALGB demonstrated only modest therapeutic effectiveness and, more importantly, a high incidence of severe toxicity and treatment-associated deaths.[112]

Total-body irradiation has been used in patients with CLL. A group of 42 patients was treated with total-body irradiation by Johnson at the National Cancer Instutute.[64] He reported 33% complete responders and 55% partial responders. These results are most encouraging. Late follow-up of these patients will be very important before total-body irradiation is recommended as a standard procedure for CLL.

Leukopheresis

Therapeutic leukopheresis has been attempted in patients with CLL. Although there may be a temporary decrease in the white blood cell count and a decrease in organ enlargement, it is not an effective method for long-term management.[42]

Course of the Disease

Except for the unusual patient who obtains a complete remission with chemotherapy or radiotherapy, the course of CLL is marked by initial improvement but not disappearance in signs and symptoms of the disease with therapy. It is rare to obtain a remission of the immunologic abnormalities with therapy. In particular, the acquired hypogammaglobulinemia usually persists from its onset to the death of the patient, and bacterial infection is the major cause of death in CLL.

The course of CLL is usually one of gradual progression. A "blast phase," such as occurs in chronic granulocytic leukemia, does not occur. Acute leukemia has been reported as a complication following the use of alkylating agents for other hematologic diseases, e.g., polycythemia vera and multiple myeloma.[7,97] It is intriguing that acute leukemia has only rarely been reported as a terminal event in CLL despite the prolonged use of alkylating agents and radiotherapy.

A small group of patients with CLL have been described who develop an aggressive lymphoma late in the course of their disease. This association is termed "Richter's syndrome" after the author who reported the first case in 1928.[100] The lymphoma is usually diffuse histiocytic lymphoma, but

Hodgkin's disease has also been described. Detailed immunologic studies have been performed on only a small fraction of these patients. The results suggest that, at least in some of the cases, the lymphoma may not represent the emergence of a second malignant clone but may actually be related to the initial B cell CLL.[55]

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CHAPTER 5

Philadelphia Chromosome–Positive Chronic Myelogenous Leukemia: Diagnosis and Treatment

Zalmen A. Arlin

Chronic myelogenous leukemia (CML) is a primary disorder of the bone marrow that leads to expansion of the granulocytic series, less commonly the megakaryocytic series, and infrequently the erythroid cells. As a result of the expansion of the elements of each of these series, characteristic signs and symptoms occur (see below). The disease has two distinct phases: one, in which essentially "normal" differentiation occurs, can be readily controlled by many chemotherapeutic agents; and an acute phase, which usually results in limited survival and cannot be readily controlled. In general, the disease is associated with a specific chromosonal abnormality, and studies of this have lead to a greater understanding of how CML evolves. CML is distinguished easily from acute granulocytic leukemia when the former is in the chronic phase. However, it may be indistinguishable from the latter when the disease presents as an acute leukemia.[2,61] This chapter is a review of the clinical characteristics of the disorder, the studies of the Philadelphia chromosome (Ph^1) that yield information about the nature of the disease, and the therapeutic options that are currently available.

Clinical Manifestations

The clinical manifestations of CML are well known. The disease occurs in both children and adults, but the median age of most large series is in the 50–60 year old age group, indicating a propensity for older patients. The clinical course is independent of age and both younger and older patients can expect similar signs, symptoms, and survival.[8] Unlike acute leukemia, in which the sudden appearance of infection or bleeding may be a prominent feature, patients with CML gradually develop nonspecific symptoms, including weakness, fatigue, bone pain, sweating, fever, and weight loss. Commonly, when patients are first seen, it is difficult for them to pinpoint when symptoms first appeared. With increasing frequency, patients in the United States are now diagnosed without any symptoms. The diagnosis is suspected on the basis of finding either an enlarged spleen on a routine physical exam, or an abnormally elevated white blood count during the course of either elective surgery or routine examination of blood counts.

On physical examination, the most common finding is the presence of an enlarged spleen and, less commonly, an enlarged liver. Generally, increased sternal tenderness may be found, and although historically considered an important physical finding, it does not carry the same weight as the more significant laboratory studies that can now be performed. Other physical signs are less commonly seen. Retinal hemorrhages may be found, but the finding of ecchymosis over the extremities is infrequent. Significant hemorrhage is uncommon in patients with CML who have not experienced traumatic injury. In instances of surgery, bleeding may be prominent, but the coagulopathy found in CML is generally not so significant that spontaneous bleeding occurs (see below). The spleen may become very enlarged and fill the entire left side of the abdomen. If this occurs, it may outgrow its blood supply, and splenic infarcts leading to severe splenic pain and tenderness may also supervene. Spontaneous rupture of the spleen

is extremely infrequent. By simple mechanical factors, an enlarged spleen of this degree may lead to diminished food intake and weight loss. Enlarged lymph nodes are extremely uncommon in chronic phase CML. More commonly, this occurs in CML when there is acceleration of the disease or during the terminal phase.[42]

Although the symptoms that have been described may appear gradually over a period of months, in general they disappear quite rapidly once treatment begins. It is quite remarkable that although a true remission of the disease (see below) rarely occurs, control of the disease is compatible with achieving a normal functional state that may last for many years.

Laboratory Findings

The most important laboratory abnormalities seen are hematologic in nature and are summarized in Table 5–1. In the peripheral blood, an increase in the granulocytic series is the most commonly found abnormality, and, in general, at the time of presentation the white count is above 100×10^8/dl. Some patients may be seen with white counts as high as 500×10^8/dl or more. Similarly, some patients may be seen with a white count of less than 20×10^8/dl and occasionally the white count may fluctuate in a cyclical pattern.[29,47,75]

TABLE 5–1 Hematologic Findings in Chronic Phase CML

Finding	Mean	Range
Hemoglobin (g/dl)	9.7	5.4–14.4
Platelets ($\times 10^8$/dl)	485	24–1400
Leukocytes ($\times 10^8$/dl)	205	20–622
Cells	**Peripheral Blood (%)**	**Bone Marrow (%)**
Neutrophils	53	42
Metamyelocytes	8	22
Myelocytes	23	19
Promyelocytes	3	4
Blasts	2	3
Basophils	3	—
Eosinophils	3	—
Lymphocytes	3	2
Monocytes	2	2
Erythroblasts	—	6

Blood findings are from Spiers et al.[85] and bone marrow findings from unpublished data.

Examination of the peripheral blood smear reveals the entire spectrum of differentiation of the myeloid series from blasts to mature neutrophils. Usually less than 3% of the cells are blasts. If more than 3% blasts are seen, this may be an unfavorable sign and herald the onset of the accelerated phase of the disease. Eosinophils and basophils are occasionally seen, and occasionally both cells may represent a significant proportion of the circulating granulocytes. It has been suggested in some reports that basophilia may also herald the onset of the accelerated phase of the disease.[17,86]

The hemoglobin usually ranges from 9 to 10 g/dl. If the hemoglobin is lower, causes such as hypersplenism, bleeding, and accelerated phase of the disease should be considered. The platelet count, in general, is elevated and may occasionally reach values as high as $3000–4000 \times 10^9$/dl/m. Severe thrombocytosis of this degree may be found at the time of diagnosis, but more frequently it is seen after splenectomy or as the disease evolves into a more aggressive phase. Thrombocytosis may represent one of the more difficult therapeutic problems in CML. The bone marrow is usually hypercellular, with preservation of few fat spaces. It is important to note the high myeloid to erythroid ratio, frequently as high as 20 to 1, as opposed to the more normal 3–4 to 1. The blast percentage is most often less than 3%. As in the case of the peripheral blood findings, a higher value may be associated with more aggressive disease.

Numerous functional abnormalities of both white cells and platelets have been described, but in general this does not translate into a higher incidence of infection or spontaneous bleeding. The prothrombin time and partial thromboplastin time, in general, are normal. Recently, a deficiency of factor V has been noted in patients with chronic phase CML.[37] When this occurs, prolongation of both the prothrombin time and partial prothrombin time may be found. An explanation for the occurrence of this abnormality remains unclear.

Classically, a low leukocyte alkyline phosphatase (LAP) level has been used as a specific means of separating CML from a leukemoid reaction and from other myeloproliferative disorders; in general, this is a typical finding in chronic phase CML.[60] However, patients may be found in whom the LAP score is normal, and it has been suggested that a rising LAP score in patients with proven CML may reflect an acceleration of the disease and transformation to an acute leukemia.[86]

Similarly, high levels of serum B_{12} and elevated, unsaturated B_{12} binding capacity are usually seen.[12] Since this reflects an elevated transcobalamin I, which is produced in the granulocytes, this does not offer a more sensitive means of monitoring the disease than monitoring the blood counts. The presence of the Ph^1 is the most important single finding establishing and confirming the diagnosis of CML in a patient with an elevated white blood count and the findings noted above. The Ph^1 is present in the majority of patients with CML, and in individual patients 100% of the bone marrow or peripheral blood metaphases that are examined are usually Ph^1 positive (Ph^1+).

Philadelphia Chromosome

The Ph^1 was first described in 1960.[58] At that time it was felt to be a deletion of the long arm of chromosome 21. Subsequently, it was shown that the abnormality actually involved a change in chromosome 22. It was at that time thought to be specific for CML, but since then it has been described in related myeloproliferative disorders and in acute leukemias. Although many investigators believe that the Ph^1 is thus no longer specific for CML,[5,40,62] careful examination of the other disorders in which it is found suggests that these diseases may all be related and originate in the transformation of a common pleuripotential hematopoietic stem cell. Until 1973, it was believed that the material from chromosome 22 was lost from the cell. With the development of banding techniques, studies by Rowley indicated that the previously described deletion of chromosome 22 was, in fact, a translocation from the long arm of chromosome 22 to the long arm of chromosome 9.[67] This raised the question of whether it was the disruption of chromosome 22 and the loss of part of its genetic material that led to CML, or whether it was the acquisition of extra genetic material by chromosome 9 that led to the disease. Subsequent studies have shown that many other translocations are seen. In all, the common feature is the translocation of the long arm of 22 to another chromosome, thus indicating the crucial importance of the break in the long arm of 22. Occasionally, complicated translocations involving three or more chromosomes have been described, and in all these cases, a translocation from chromosome 22 is always seen. These unusual and complicated translocations represent approximately 10% of the patients with Ph^1+ CML.[27] Thus far, no clinical significance appears to be associated with the nature of the translocation encountered (Figure 5–1)[72,82]

The Ph^1 is found in myeloid, erythroid,[13] and megakaryocyte[41] cells. In patients with chronic phase CML, lymphoid cells stimulated to cell division by commonly used mitogens do not contain the Ph^1. However, the Ph^1 can be found in approximately 20%–30% of cases of blastic transformation of CML, in which the cells generally have lymphoid morphology, react with common acute lymphoblastic leukemia (ALL) antigen, and contain terminal deoxynucleotidyl transferase. Although generally these cells have proved to be "null" by conventional surface marker phenotypic studies, cases have recently been reported in which the cells form rosettes with sheep red blood cells, thus indicating that occasionally these T cells may be also involved.[64] In other cases with lymphoid morphology, it has been demonstrated that the cells contain intracytoplasmic immunoglobulin and are therefore felt to be pre–B cells.[48] Studies by Fialkow et al., using the system developed in glucose-6-phosphate dehydrogenase (G6PD) heterozygotes, suggested that T cells in general do not participate in the leukemic population, as evidenced by their nonclonal nature in these

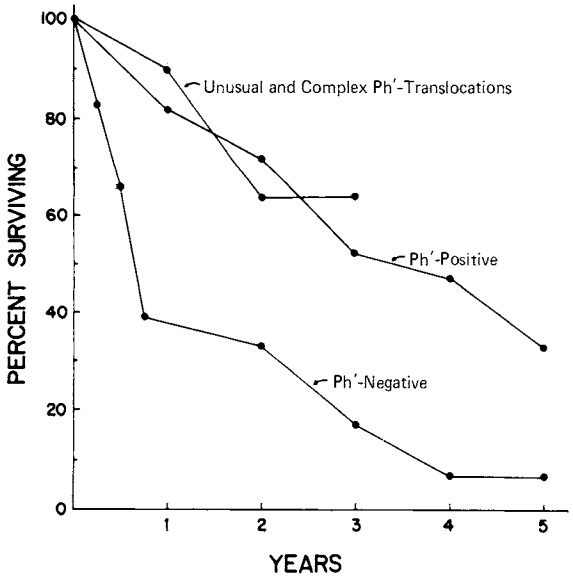

FIGURE 5–1 Survival of patients with CML and unusual and complex Ph^1 translocations compared with survival of patients with the standard translocation and Ph^1– CML. (From Sonta and Sandberg.[82])

TABLE 5–2 Cytogenetic Studies During Chemotherapy in a 45,X/46,XX Mosaic

Status of Chemotherapy	Ph^1 Chromosome Status in Bone Marrow (no. positive/total)	Chromosome Numbers			
		45,X,Ph^1−	45,X,Ph^1+	46,XX,Ph^1−	Total
Prior to DAT,[a] cycle 1	30/30	0	15	0	15
Prior to DAT, cycle 2	5/30	4	4	12	20
Prior to DAT, cycle 3	3/30	2	3	13	18
Prior to maintenance	1/30	0	0	9	9

Adapted from Chaganti et al.[9]
[a] DAT: daunorubicin with cytosine arabinoxide, and 6-thioguanine.

patients.[22,23,24] This was recently confirmed by Kearney et al.[46] They stimulated the peripheral blood T lymphocytes from a patient with CML and noted the absence of the Ph^1. They further suggested that some B cells probably do participate in the leukemic process, as evidenced by their clonality. The bone marrow fibroblasts are not participants in the clone.[49] Thus, bone marrow fibrosis, which is frequently seen in the latter stages of the disease, is thought to be reactive rather than a derivative of the malignant population.

At time of diagnosis, virtually all the metaphases examined in either the peripheral blood or the bone marrow contain the Ph^1. In a small minority of patients both Ph^1+ and Ph^1-negative (Ph^1−) cells may be seen.[6,26,70] The presence of Ph^1− cells in this small group of patients may have clinical significance, since it has been suggested that patients with some Ph^1− cells have an improved survival over those who are entirely Ph^1+. Of greater significance is the possibility that these may represent normal residual hematopoietic cells that may serve as a reservoir to repopulate bone marrow after effective treatment of leukemic cells has taken place.

Considerable controversy previously existed with respect to the nature of the Ph^1− cells. Early studies by Singer et al.[78] suggested that the Ph^1− cells are probably also participants in the malignant process. This is suggested by their studies of the G6PD isoenzyme system, which demonstrated the same isoenzyme in the Ph^1− cells as occurred in the leukemic cell population. This finding could be explained by a two-step hypothesis for the pathogenesis of CML:[25] namely, one event occurs that leads to the transformation of a pleuripotential cell into a malignant cell, and a second event occurs subsequently that leads to the acquisition of the Ph^1 abnormality. Thus, not all the cells that arise from the initial leukemic event may have been exposed to the second event that induced the Ph^1. This has extremely important implications for treatment. If one contemplates efforts at eradication of malignant cells, it is imperative that normal residual cells be present to reconstitute the bone marrow after the leukemic cells have disappeared. However, efforts at unmasking Ph^1− cells would be pointless if one simply replaces leukemic Ph^1+ cells with leukemic Ph^1− cells. A more recent study by Singer et al.,[79] in collaboration with our group, in a patient who was heterozygous for the G6PD locus and who underwent cytogenetic conversion from the Ph^1+ to the Ph^1− state indicates that indeed the Ph^1− cells in her case represented normal hematopoiesis. Examination of granulocytic colonies and erythroid colonies as the patient was undergoing conversion to the Ph^1− state indicated restoration of nonclonal erythropoiesis and granulocytopoiesis. This supported the view that the Ph^1− cells are normal. Further evidence for this was seen in a second patient, who was mosaic for 45,X and 46,XX.[9] As seen in Table 5–2, the patient's malignant clone, which contained the Ph^1, occurred in the cell that was constitutionally 45,X. Subsequently, as treatment progressed, cells that were 46,XX and Ph^1− appeared, as well as cells that were 45,X and Ph^1−. Although an argument could be made that the latter cells may have participated in the malignant clone, this could not be said for the 46,XX Ph^1− cells because of the already established clonal nature of CML.

Treatment

Many review articles have been written in the past few years that have described in detail the current standard treatment of CML. The common thread that runs through these reviews is the observation

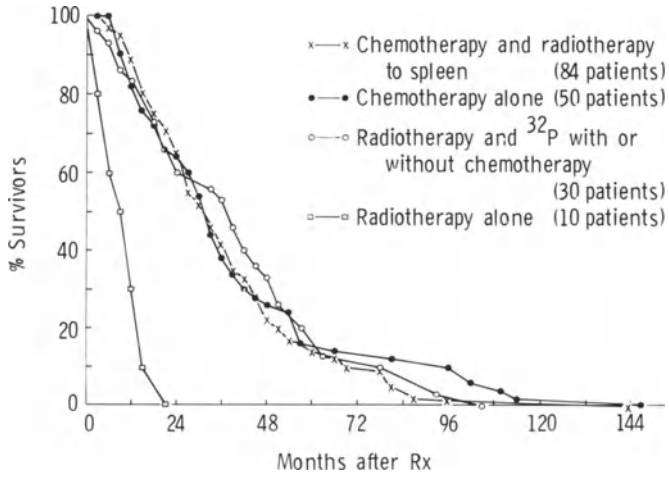

FIGURE 5–2 Survival in CML with single-agent chemotherapy compared with radiotherapy. These results are typical of many large series and not appreciably better than Minot's series (54). (From Monfardini et al.[56])

that very little progress has been made in the treatment of CML.[15,53,56] Indeed, many authors have compared their experience with the original review of CML by Minot et al. in 1926,[54] and have concluded that survival curves since then have only marginally improved (Figure 5–2). In part, it is the unique nature of CML that has hampered the introduction of new therapies. As described above, patients with CML in the untreated state are generally not very symptomatic and their disease can be controlled very readily with very simple measures (see below). Since the white cells in CML function fairly normally, as do the red cells and platelets, control of the disease is compatible with an open-ended survival that in some patients may last beyond a decade. It is difficult for clinicians to come to terms with the need to consider potentially toxic treatment for healthy-appearing patients.

This contrasts with the experience in acute leukemia, in which survival in the untreated state is measured in a few months and the "controlled" state is usually associated with problems of bleeding and recurrent infection. Thus, efforts at inducing true remission of acute leukemia are acceptable and mandatory and have yielded improved survival of ALL, with cure rates in both adults and children now approaching 50%. Although the results in acute myelogenous leukemia are less dramatic, true remissions are now commonplace, and although survival on the average is only approximately 1 year, a small group of 15%–25% may achieve survival that exceeds that of patients with CML. The recognition that survival in CML has not changed appreciably over the past several decades has led a number of centers to offer innovative approaches to younger patients, in whom a median survival of 4 years does not seem to offer very much. Thus, it has been possible recently to examine the effectiveness of bone marrow transplantation and intensive chemotherapy in changing the natural history of CML.

Treatment of Chronic Phase

Prior to the advent of systemic chemotherapy, the traditional treatment of CML was splenic irradiation. This was usually delivered in daily fractions of up to 100 rads, and in most instances it led to a rapid reduction of the size of the spleen and the return to the normal range of elevated white blood cell and platelet counts. As with alkylating agents, which were developed later, control of the disease could be achieved for periods of months. With the regrowth of the size of the spleen and elevation of the white blood count, additional radiotherapy could achieve control once again. This sequence could be repeated several times, and patients could remain fairly asymptomatic through the anticipated life expectancy of 120 or more weeks.

It is interesting to note that the delivery of daily fractional doses, totaling 1500 rads over 2 weeks, was much more effective in achieving control than was the administration of 1500 rads in one or two fractions. This observation lends support to the hypothesis that the mechanism of action of splenic irradiation was the destruction of the hematopoietic stem cells that circulate through the spleen: greater numbers of stem cells are exposed to radiation delivered over many days than during only

one exposure. Similarly, it also tends to discount the hypothesis that the irradiated spleen releases an inhibitor of myeloid proliferation into the circulation, which subsequently inhibits the production of cells in the marrow. The latter cannot account for the differences seen when the rate of delivery of radiation is varied. Radiotherapy, although generally safe, occasionally can lead to long-term pancytopenia. It is partly as a result of this occasional complication, and partly because chemotherapeutic agents can be given more easily without the need for radiotherapy units, that chemotherapy has generally replaced splenic irradiation as the treatment of choice for CML.

Generally, the chronic phase of CML is treated with single oral agents. The drug preferred by most clinicians is busulfan, an oral alkylating agent.[38] In a large trial that compared splenic radiotherapy with busulfan, the chemotherapy-treated patients had a survival advantage.[53] Although it has now been used for approximately 20 years, there is no reason to believe that busulfan is better for CML than the other drugs that are currently available. Few trials have been undertaken that compare busulfan to other agents, but when it has been done, there was no evidence that busulfan is superior or that it leads to prolonged survival. It should be pointed out that busulfan is administered easily, is generally well tolerated by the patient, and may occasionally give long periods of control without the need for continuing treatment[18] or with the need for only very minimal treatment.

Therapy generally begins with busulfan 4–10 mg daily. As the blood count is reduced, the dose of busulfan is reduced sequentially. It is important to stop therapy when the white blood count reaches 25–50 $\times$ 10^8/dl. There is a risk of bone marrow aplasia when busulfan is administered for too long. However, by stopping treatment when the count is still somewhat elevated, this risk is essentially negligible. Since there is no serious risk to having the white count rise back to over 100 $\times$ 10^8/dl, stopping therapy at the level noted above is an important safety factor. After the white count stabilizes, patients may resume low-dose busulphan, usually 2 mg once or twice weekly, which may maintain the blood count in the region of 10–30 $\times$ 10^8/dl. It is imperative that the white count never fall below this range for the reasons stated above, and it is much safer to permit the count to approach 30 $\times$ 10^8/dl than to "fine tune" it in the 5–10 $\times$ 10^8/dl range. Since there is no evidence that maintaining the white count in the normal range leads to a superior survival over its maintenance at the higher range, it is always safer to undertreat than to overtreat.

Busulfan is not without side effects. Its main nonhematologic side effects include brown discoloration of the skin. Amenorrhea is common, and dysplastic changes in vaginal and cervical mucosa have also been noted. Azoospermia has also been seen shortly after beginning busulfan. Much has been written about the so-called busulfan lung, but this phenomenon is rare. Patients may develop cough, fever, dyspnea, and interstitial pulmonary changes. Frequently this may progress, and the patient may die of respiratory causes. Other conditions that have been seen include cataract formation and neurologic degenerative disease in patients who have been on busulfan for long periods.

Hydroxyurea is an oral antimetabolite that acts by interfering with DNA polymerase.[28] It is primarily used in treating patients with chronic phase CML. Although it is not as commonly used as busulfan, it is considered the treatment of choice at the Memorial Sloan Kettering Cancer Center. Treatment generally begins with 1–3 g hydroxyurea daily. This usually reduces the blood count over a period of days. Although rapid lowering of the blood count is generally not important, this contrasts with busulfan therapy, in which lowering the leukocyte count may require several weeks. As the white count falls, the dose of hydroxyurea is reduced, but, unlike busulphan, it is virtually impossible to eliminate totally the need for continuing treatment. In general, patients must remain on maintenance doses that may vary between 0.5 and 3 g daily. The usual maintenance dose in most patients, however, is approximately 1–2 g/day. As in the case of treatment with busulfan, efforts should be made to keep the white count between 10–30 $\times$ 10^8/dl.

The main advantage to using hydroxyurea over busulfan is that it is virtually impossible to induce aplasia with hydroxyurea. When the dose has been inadvertently taken for too long, rapid recovery of the blood counts occurs within a few days of stopping the hydroxyurea. The other side effects seen with busulfan are also not seen, and only rarely do patients complain of minor scaling or redness of the skin. We believe that this remarkable absence of side effects should make hydroxyurea the treatment of choice for this disease. Although there is a theoretical risk that busulfan, as an alky-

lating agent, may induce the emergence of blastic clones, there is no evidence that the survival of patients receiving hydroxyurea is better than with busulfan.[68] These two drugs remain the mainstay of treatment. It should be pointed out, however, that many other agents can also be used,[19] and it is our opinion that many alkylating agents and antimetabolites may also be effective and, when given in sufficiently high doses, achieve the same control.

Whether busulfan or hydroxyurea is given, it is necessary to administer fluids and allopurinol, 300–600 mg/day. This will obviate any problems that may result from tumor lysis and the production of uric acid. It is also interesting to note that, unlike the treatment of acute leukemia, which requires hospitalization in order to achieve control of the disease, treatment can be started and administered throughout the chronic phase on an outpatient basis. It has been shown that there is a higher risk of hemorrhage when the white count exceeds 100×10^8/dl in acute myelogenous leukemia. However, the risk of this occurring in CML is significantly lower. Thus, the urgency to lower the count quickly in most cases does not exist.

Probably the most difficult problem encountered in treating chronic phase CML is the treatment of thrombocytosis. It has been suggested that since the platelets do not function very well in CML, it is permissible to allow the platelet count to rise over 10^6/dl or more without the patient developing significant risk of emboli and bleeding. Although this occasionally may be true, more commonly, symptoms develop in patients when the platelet value approaches this level. Dizziness, headaches, confusional states, and stroke may occur when the platelet count rises above this level. Thus, it is imperative that the platelet count be reduced in these patients as soon as possible. In patients who are receiving hydroxyurea, the dose of the drug can be increased, accepting a white count of approximately 5×10^8/dl. This frequently may be sufficient to control the platelet count. Needless to say, this is not as readily acceptable for patients who are on busulfan, since lowering the white count to 5×10^8/dl with the latter drug may lead to a potentially dangerous clinical situation that may result in prolonged aplasia. Thus, when there is a need to increase therapy to control platelets, we favor the use of hydroxyurea.

Occasionally, hydroxyurea may not resolve the thrombocytosis. This is evidenced by a decrease in the granulocyte level to less than 10^8/dl without concomitant reduction of the platelet count below 1×10^{11}/dl. Alternative therapies in this situation include the administration of an intravenous thiotepa in doses of 1–1.5 mg/kg/week until control is achieved, and then maintenance with weekly thiotepa according to the level of the platelet count. Another option is the administration of melphalan, which it has been claimed may be more selective against the megakaryocyte line.[4] Melphalan is usually started at a dose of 6–10 mg p.o., daily with gradual reduction of the dose as the platelet count begins to fall. Frequently, a daily maintenance dose of 2 mg is all that is required to achieve adequate control. Uracil mustard is an oral alkylating agent that also has been used effectively to treat patients with thrombocytosis.[63,76] A dose of 1–2 mg/day is given daily for 14 days, following which the course can be repeated as needed, or patients may receive continuous therapy at a dose of 2–7 mg/week. Patients who receive the intermittent schedule may not require a repeat course for as long as 3 months. Another alternative may be the administration of repeated doses of ^{32}P at 3–4 week intervals. This often controls the situation when the measures noted above have not been successful. A dose of 5 μCi given for 2–3 doses may be all that is required. In the emergency situation, it may be necessary and, indeed, desirable to perform a plateletphoresis. This procedure can reduce the platelet count to one-half the pretreatment value over a period of 3 hours, very often leading to immediate resolution of the symptoms noted above. However, since this reduction of the platelet count is short-lived, it is imperative simultaneously to start intravenous alkylating agents, such as thiotepa, to achieve more long-term control.

There are special circumstances in which splenectomy might be beneficial.[30] First, the patient may have an unusually large spleen that mechanically prevents an adequate oral intake. Removal of the spleen frequently can correct the weight loss by improving food intake. Anemia and thrombocytopenia may become a prominent feature of the disease due to hypersplenism. Removal of the spleen may correct this problem. Blastic transformation may occur primarily in the spleen; it is characterized by rapid growth of the spleen, associated with a disproportionale increase in blasts in the peripheral blood. Splenectomy, followed by chemotherapy, may restore the disease to the

chronic phase. However, in patients who are fully blastic with the transformation occurring primarily in the bone marrow and only secondarily leading to an enlarged spleen, splenectomy is not indicated. In the latter case, the short survival that results from the transformation precludes, in most instances, a beneficial effect of splenectomy. A patient whose blastic phase is following a more indolent course may still be a candidate for splenectomy if the indications noted above are present.

True Remission

In view of the poor survival associated with conventional therapy, two new approaches have emerged in an attempt to achieve cure of the disease. The first employs sublethal doses of chemotherapy and is directed at eradication of the Ph^1- cells with restoration of the normal hematopoiesis that presumably persists in most patients with CML. The second employs supralethal doses of radiotherapy and chemotherapy with reconstitution of the bone marrow with either syngeneic or allogeneic bone marrow.

The first program using sublethal doses of chemotherapy was initiated at Memorial Hospital, New York City.[16] From 1970 to 1976, 37 adults with Ph^1+ CML received treatment on the L-5 protocol (Table 5–3). Treatment consisted of splenic irradiation followed by splenectomy and three courses of cytosine arabinoside and 6-thioguanine, followed by L-asparaginase, followed by sequential maintenance chemotherapy. Details of this program have been published previously.[16] Of the 37 patients, 29 were evaluable for response. Five of the 8 unevaluable patients had undergone blastic transformation prior to the beginning of chemotherapy, and 3 additional patients were excluded from analysis either because they did not follow the protocol or because bone marrow studies were not evaluable for cytogenetic response. Of the 29 evaluable patients, 12 had a significant reduction in the percentage of Ph^1+ cells in their marrow; 7 of these had complete responses, with reduction of the Ph^1+ cells to undetectable levels, and 5 others had reduction to less than one-third of the pretreatment value. The median duration of response in most cases was very brief. Of these 12 patients, 8 relapsed in less than 4 months; 2 patients, however, had a sustained reduction in Ph^1+ cells for more than 3 years. One of these remained continuously Ph^1- for the last 1½ years, of his life and was Ph^1- at the time of his death from metastatic adenocarcinoma of the lung.

TABLE 5–3 L-5 Protocol: Treatment Plan for Induction of True Remission in Ph^1-Positive CML

1. Radiotherapy to spleen
2. Splenectomy
3. Intensive chemotherapy with cytosine arabinoside and 6-thioguanine
4. L-Asparaginase
5. Hydroxyurea

From Cunningham et al.[16]

It is interesting to note that even though the duration of true remission was brief, those patients who responded had a statistically significant longer survival than those who did not respond (Figure 5–3). It is therefore possible to conclude that the treatment effected a change in the natural history of the disease such that, although a true remission was not sustained, early transformation may have been prevented, thus shifting the survival curve to the right. Early intensive treatment may have eliminated small numbers of cells exhibiting additional aneuploidy.[83] An alternative explanation for this finding is that treatment did not affect the disease but may have selected out a group of patients who, by virtue of their ability to respond, had an improved survival. There is a precedence for this view since it has been demonstrated that patients who were mosaic at the time of diagnosis (i.e., had bone marrows that contained both Ph^1+ and Ph^1- cells) had a better survival than those patients who had only Ph^1+ cells.[16,25,33,70] The L-5 protocol may thus simply unmask an additional group of patients who are mosaic as well. Others have recently confirmed that chemotherapy with or without splenectomy may induce true remissions, but, because of the small numbers treated, have not confirmed the survival advantage of responding.[77,80]

Since the L-5 protocol failed to cure the vast majority of patients and may have been curative in only one patient, an alternative chemotherapy regimen was started in 1977.[35] The sequence of chemotherapy in protocol L-15 is outlined in Figure 5–4; it is a modification of the L-14 protocol used at that time for induction of remission in adult acute nonlymphoblastic leukemia. In essence, daunorubicin is added to the combination of cytosine arabinoside and 6-thioguanine, and in-

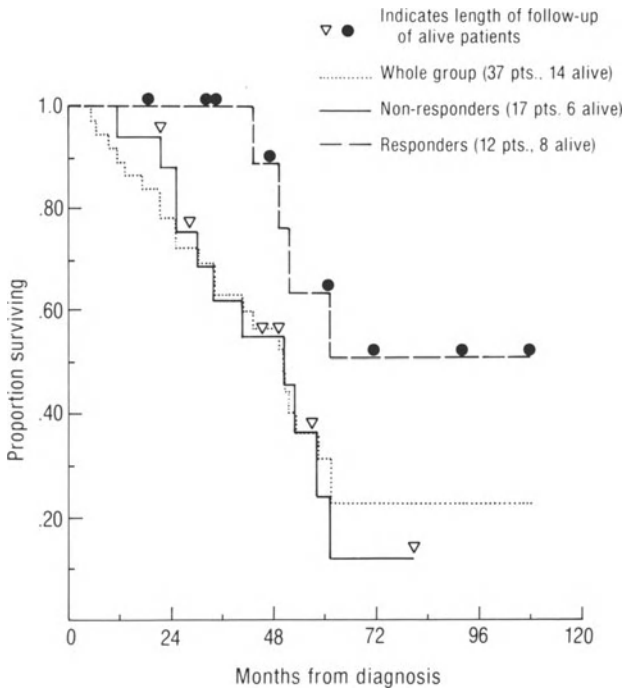

FIGURE 5–3 Results of L-5 protocol for treatment of Ph¹+ CML: survival of patients achieving a true remission compared with survival of those failing to respond. (From Cunningham et al.[16])

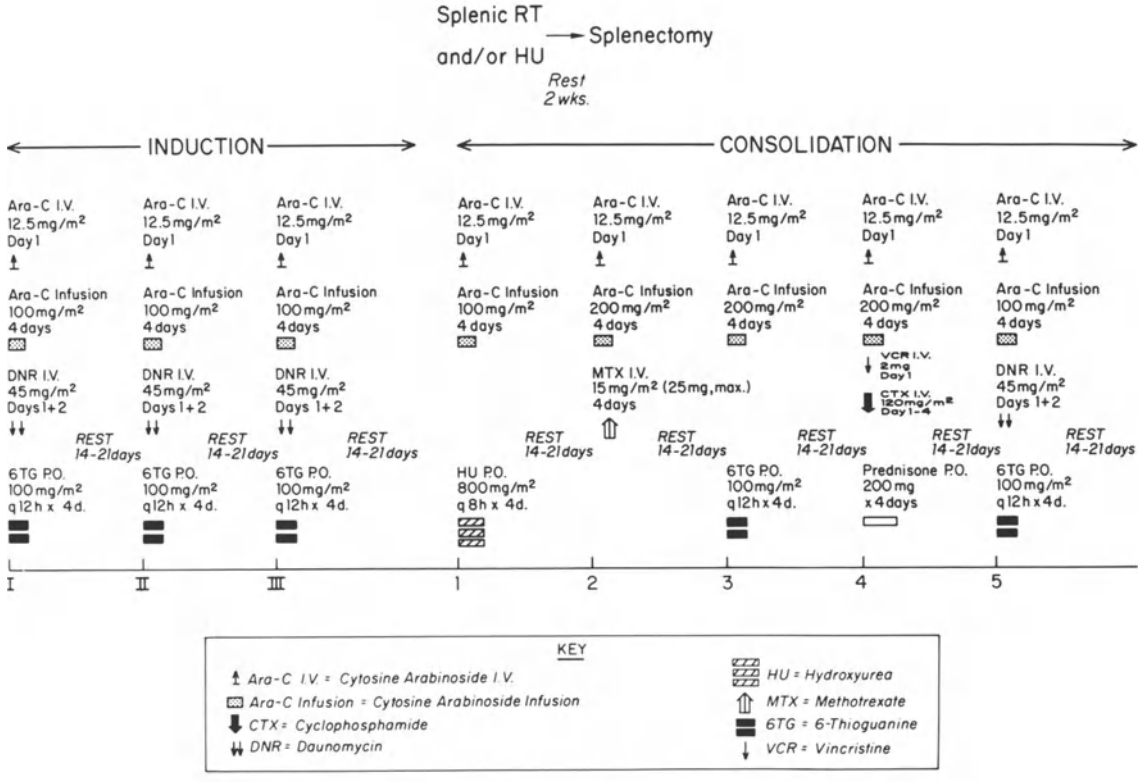

FIGURE 5–4 L-15 protocol for induction of true remission in Ph¹+ CML. (From Goto et al.[35])

TABLE 5–4 True Remission of CML in Two Protocols

Outcome	L-5 No.	L-5 %	L-15 No.	L-15 %
Evaluable patients	29		42	
Complete remissions	7	28	14	33
Partial remissions	5	14	6	15
Total remissions	12	41	20	48

stead of the cytosine arabinoside being administered as an intravenous injection every 12 hours, it is given as an infusion. The reason for the addition of daunorubicin is the observation that the two longest responders on the L-5 protocol had received an additional course of intensification with daunorubicin and cytosine arabinoside, and the three-drug regimen yielded a higher remission rate in acute nonlymphoblastic leukemia than cytosine arabinoside and 6-thioguanine. It was hoped that a similar improvement in remission rate of CML could result from this change. The consolidation sequence was further intensified since the rather gentle maintenance chemotherapy of the L-5 protocol failed to prevent relapse.

There were 42 patients treated with the L-5 protocol: 20 responded and 22 failed to respond. Of the 20 responders, 14 had reduction of their Ph^1+ cells to 0%. Thus, the L-15 protocol only marginally increased the total remission rate (from 41% to 48%) and the complete remission rate (from 28% to 33%) (Table 5–4). Unfortunately, as was the case with the L-5 protocol, the remissions were short lived, lasting a median of only 4 months. Furthermore, in this series, the beneficial effect of responding was no longer evident. The median time to entry into blast crisis was similar for both responders and nonresponders. Seven of the responders and seven of the nonresponders developed blastic transformation at 32.6 months and 33.8 months, respectively. At the present time, the median survival has not yet been reached in either group, but the Kaplan-Meier plot revealed no statistical difference in the respective survival (Figure 5–5).

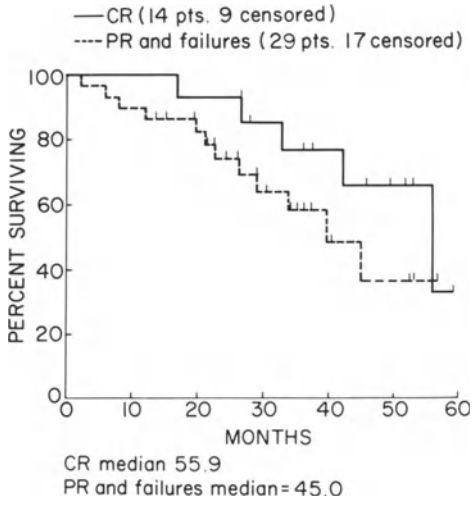

FIGURE 5–5 Results of L-15 protocol for treatment of Ph^1+ CML: achieving a true remission compared with survival of those failing to respond. (From Goto et al.[35])

In general, treatment was well tolerated. Although patients frequently developed pancytopenia, hospitalization was required in only 60% of cases for fever, and this was usually resolved within 7–10 days on intravenous antibiotics. Unfortunately, one patient in this series died as a result of a subphrenic abcess. This patient represents the only death resulting from treatment on both the L-5 and L-15 protocols. In view of the neutropenia and thrombocytopenia that occurred, further intensification of this therapy does not seem possible without further increasing the risks to the patient.

This trial suggests that the administration of sublethal doses of the chemotherapeutic agents given in the manner described above cannot eradicate the Ph^1- containing clone in virtually all patients treated. It further suggests that until other, more selective agents are developed, this type of aggressive approach to cytotoxic chemotherapy will not be successful. In this regard, it may become necessary to evaluate newer agents such as those that may induce terminal differentiation of myeloid cells. By forcing the clonogenic cells to differentiate, the proliferative advantage now held by leukemic cells may be overcome.[69]

That eradication of chronic phase CML is possible by means of supralethal doses of radiotherapy and chemotherapy has been demonstrated by the Seattle group.[21] In their study 12 patients with chronic phase CML who had identical twins were treated with dimethylbusulfan, 5 mg/kg i.v. followed by cyclophosphamide 20 mg/kg/day for 2 days and total-body supralethal irradiation therapy. In addition, the patients received methotrexate intrathecally and bone marrow from their syngeneic sibling was administered within 24 hours

of the radiotherapy. Bone marrow engraftment occurred in all 12 patients, and in all cases bone marrow studies done on recovery of blood counts revealed the absence of the Ph^1. Subsequent follow-up of these patients revealed relapse with reappearance of the Ph^1 in 3 patients. One patient in this series died as a result of interstitial pneumonia. At time of his death, he was still Ph^{1-}. The remaining patients all have remained Ph^{1-} for 7–55 months after transplantation. Thus, treatment as administered by the Seattle group can successfully eradicate the Ph^{1+} clone while the patient is still in the chronic phase of the illness.

This positive report has led several centers to begin exploring the use of allogeneic transplantation for patients in either the accelerated phase or chronic phase of CML. McGlave et al. described their experience with this approach in nine patients with accelerated phase CML.[52] All nine patients had evidence of engraftment and restoration of blood counts to normal. In addition, they all had complete disappearance of the Ph^1, and one patient also had disappearance of myelofibrosis. Two of the nine patients died of graft-versus-host disease while still in remission of the CML. Seven patients were alive and still in complete remission of their CML after 45–270 days. Goldman et al. treated 12 patients in a similar manner using allogeneic bone marrow.[34] Successful engraftment occurred in all 12 patients, but 4 patients died of either infection or transplant-related complications. All of the patients in whom follow-up could be performed revealed restoration of a Ph^{1-} marrow. Champlin et al. similarly treated patients with either chronic or accelerated phase CML, and six of their eight patients have remained in remission with normal bone marrow cytogenetics 3–21 months after transplant.[10,11] Others have made similar observations.[20,83]

These studies indicate that the disease is curable with the combination of chemotherapy and radiotherapy possible only in a transplant setting. The report by Oblon et al. further supports the concept that bone marrow transplantation may result in clearing of myelofibrosis.[59] Transplantation is not without risks, and a small but real mortality occurs as a result of the procedure. However, since the majority of deaths occur not from recurrent leukemia, but from transplant-related problems, further improvement in this technique should offer a real chance for cure in the majority of patients with CML.

Blastic Transformation

Were it not for the emergence of the acute leukemic phase, patients with CML could live a normal life span with little impact on their normal functional ability. However, in the majority of patients, the chronic phase is followed by acute leukemia that, in most instances, is very resistant to treatment.

Although the diagnosis of blastic transformation requires the appearance of increased numbers of blasts and promyelocytes in either the bone marrow or the peripheral blood, patients whose disease is entering a period of transition develop characteristic signs and symptoms. The list in Table 5–5 was collected from a series of 73 patients reviewed by Rosenthal et al.[65,66] Coincident with the development of these symptoms is a change in the hematologic findings. There is a progressive fall in the hemoglobin and a simultaneous progressive fall in the platelet count. Of special interest is the patient who previously had severe thrombocytosis and whose elevated platelet count is more easily controllable. Peripheral smears usually are characterized by the presence of increased Pelger-Huet cells, increased nucleated red blood cells, and occasionally increased basophils (Table 5–6). Myelofibrosis may be present in as many as 40% of patients and may occur prior to blastic infiltration of the marrow. However, as has been shown previously, virtually 100% of patients who develop myelofibrosis undergo blastic transformation

TABLE 5–5 Signs and Symptoms of Blast Crisis

Sign/Symptom	Patients Affected (%)
Fever	60
Musculoskeletal pain	53
Abdominal enlargement/pain	38
Malaise	66
Dyspnea, cough	38
Nausea, vomiting	47
Weight loss	29
Hemorrhage	18
Infection	8
Ankle edema	8
Hepatomegaly	52
Splenomegaly	85
Adenopathy	5

Adapted from Rosenthal et al.[65]

TABLE 5–6 Peripheral Blood Abnormalities at Blast Crisis

Abnormality	Patients Affected (%)
Pelger-Huet cells	18
Abnormal erythrocyte morphology	31
Nucleated erythrocytes (range per 100 leukocytes, 1%–42%)	48
Eosinophils ≥ 5% (range 5%–18%)	7
Basophils ≥ 5% (range 5%–65%)	48

Adapted from Rosenthal et al.[65]

within 1 year of its documentation.[36] Cytogenetic evaluation documents the emergence of clones with additional abnormalities in 70%–80% of patients.[90] These abnormalities either precede or coincide with bone marrow or peripheral blood evidence of transformation. The most common abnormalities seen are shown in Table 5–7.

In order to establish that the disease has evolved into the acute phase, it is necessary to demonstrate an increase in the proportion of blasts and promyelocytes in both the blood and bone marrow. At the present time, the minimum requirement, according to the criteria established by the Acute Leukemia Group B (ALGB), is a total of 30% blasts and promyelocytes in the blood.[45] Equally acceptable is a minimum requirement of 50% in the marrow. In most instances, the difference between these two values is probably not of great clinical significance. An increase in blasts above the normally found 3% in the bone marrow is distinctly abnormal. It may occasionally occur in some patients with chronic phase CML who are recovering from intensive chemotherapy. However, when patients are receiving single-agent chemotherapy on a continuous basis, the finding of even 10% blasts in the bone marrow is ominous and indicates that the disease is no longer associated with an open-ended survival; aggressive therapy may be required to restore the disease to the chronic phase.

TABLE 5–7 Chromosomal Changes in Blast Crisis

Chromosome	Percentage
Trisomy 8	44.7
2Ph[1]	42.7
i(17q)	26.8
+19	13.0

Adapted from Whang-Peng and Knutsen.[90]

Numerous programs have been developed over the years in an effort to eliminate the acute leukemic phase when it develops and to restore the chronic phase of the disease. In general, these programs have not been effective. However, for over a decade it has been recognized that some patients will respond to treatment with vincristine plus prednisone combinations, and that in most instances this occurs in patients whose acute leukemia morphologically resembles ALL. Terminal deoxynucleotidyl transferase (Tdt) is a specific DNA polymerase found in thymocytes. This enzyme has since been found in the cells of virtually all cases of ALL, only a minority of patients with acute nonlymphoblastic leukemia, and more recently in approximately 20%–30% of patients with blastic phase CML.[51,73] It has now also been shown that the patients with blastic CML whose cells are Tdt positive (Tdt+) are the ones whose disease ultimately responds to vincristine plus prednisone.

Additional studies by Janossy et al. have indicated that the cells have the same common ALL antigen as do patients with ALL.[44]

Marks et al. treated 22 patients with blastic CML whose disease was also characterized for the presence or absence of Tdt.[50] Of the 13 patients whose cells were Tdt+, 8 achieved a remission of their acute leukemia with a return to the chronic phase, whereas none of the 9 patients whose cells were Tdt negative (Tdt−) responded. Furthermore, Tdt proved to be a more reliable indicator of responsiveness to vincristine plus prednisone than the morphologic appearance of the leukemic cells. In this series, 6 of the 13 patients with myeloblastic morphology were Tdt+, and of these, 3 had a complete response. The 2 patients with lymphoid morphology who were Tdt− failed to respond.

Although in the study of Marks et al. patients with Tdt+ blast cells responded to treatment as opposed to those who did not, this did not translate itself into an appreciably longer survival.[50] Responders in that series lived a median of only 20 weeks, whereas the nonresponders lived a median of 11 weeks.

We have recently used the Tdt assay to select patients with acute transformation of CML for treatment with an intensive chemotherapy pro-

gram currently in use for adult lymphoblastic leukemia. Therapy consists of five weekly injections of vincristine given simultaneously with daily prednisone for a period of 35 days. Doxorubicin is also added to therapy and given in midcycle. Additional intensification therapy usually follows with alternating cycles of methotrexate with cytosine arabinoside or cytosine arabinoside with 6-thioguanine. Ultimately, patients receive an eradication schedule that consists of sequential administration of vincristine plus prednisone, doxorubicin, 6-mercaptopurine plus methotrexate, and actinomycin D. This sequence is repeated every 11 weeks, and on alternate cycles BCNU plus cyclophosphamide replaces the doxorubicin. The rationale for this type of approach has been described previously.[1] Of the 28 patients treated with this protocol, 71% achieved a remission, and the median survival of the responders was 13 months.

This trial emphasizes the clinical relevance of performing the Tdt determination in patients with blastic phase CML. Patients who are Tdt− survive only briefly, usually less than 6 months compared to the Tdt+ patients, whose survival averages greater than 1 year. It should be noted, however, that none of the Tdt+ patients treated in this way have been cured of their disease. For this reason, eight of the younger patients whose siblings were HLA compatible were offered the opportunity of a bone marrow transplant. Although seven of the eight patients have subsequently died of graft-versus-host disease and interstitial pneumonia, one patient is currently free of all evidence of both chronic and acute leukemia. A second patient, reported elsewhere,[89] whose disease was approached in a similar manner had a similar result. Thus, eradication of the acute leukemic phase is potentially possible in this group of patients once the acute leukemic clone has been reduced to low levels by effective anti-ALL therapy.

Unfortunately, the acute leukemic phase of the majority of patients is characterized by cells that lack Tdt. Thus, these patients will not respond to anti-ALL therapy and require alternative measures. Although a number of programs have been devised, there is no clear evidence that any one is superior.[3,14,31,39,84,88] It is true that various reports have indicated a survival of up to 9 months in patients with acute phase CML, but since most of these programs have contained vincristine plus prednisone, and since the Tdt+ cases were not distinguished, it is difficult to determine how much, if any, improvement in survival occurred in the Tdt− cases. Our own unpublished experience with daunorubicin in combination has revealed a general refractoriness to treatment, with a remission rate of less than 10%. We have recently explored the use of the new acridine derivative amsacrine in combination and have found a somewhat better remission induction rate: 3 of 12 patients (25%) achieved a complete remission. In spite of this, however, survival is very short, even in the responders, since the blastic clone usually recurs within a period of months.

When either Tdt+ or Tdt− acute leukemia returns and alternative regimens are not available, it is reasonable to attempt to control the elevated blood counts with antimetabolites, such as hydroxyurea. When this is done, it is necessary to give sufficient hydroxyurea to control the elevated white cell count without inducing life-threatening thrombocytopenia. Occasionally, it is possible to maintain fairly normal blood counts for long periods of time, and we have seen rare cases in which control is possible for longer than 1 year. As a general rule, in view of the lack of responsiveness of Tdt− cases to conventional therapy, it is appropriate to consider the administration of phase I or II agents to this group. Only through the evaluation of new agents will a clearly effective treatment for this phase of the disease be found.

Occasionally, patients may develop some of the symptoms of blastic crisis without overt evidence of an increase in blasts plus promyelocytes in either the bone marrow or the blood. Specifically, patients may develop fevers, sweats, bone pain, and lymphadenopathy. When this occurs, it is possible that transformation is first occurring in extramedullary sites. When these symptoms are associated with a sudden increase in the size of the spleen and the presence of more blasts plus promyelocytes in the peripheral blood than in the bone marrow, the spleen may be the site of acute transformation.[55] When adenopathy is the prominent feature, aspiration of the lymph node material may indicate that the lymph nodes are the site of transformation. Finally, patients may occasionally develop severe headache and cerebrospinal fluid evidence of pleocytosis. In this case, examination of cytocentrifuge preparations may indicate that the spinal fluid is the primary site of transformation.

Each of these specific clinical situations requires therapy that is distinct from that which is given

to patients in whom the transformation has occurred in the bone marrow. In patients with lymphadenopathy that is either painful or causing symptoms of venous or lymphatic destruction, local radiotherapy is the treatment of choice. This is true whether the adenopathy is due to infiltration with Tdt+ blasts cells or Tdt− blast cells, or simply to chronic phase CML cells preferentially growing in nodal areas. Since the Tdt+ cells may also respond quite well to anti-ALL therapy, this should be given simultaneously even if the bone marrow is free of the acute leukemia. Since effective therapy of Tdt+ blastic transformation is not currently available, it is reasonable to continue to treat the patient with a single oral agent. As an alternative, the administration of amsacrine in combination might be tried. It has been established that occasionally intensive chemotherapy can eradicate subclones with additional cytogenetic abnormalities, and this may be true even in the Tdt− cases. Thus, although there is no evidence that additional chemotherapy for Tdt− blastic transformation that has occurred may be beneficial, this remains a possibility. The demonstration that the nodes contain chronic phase CML with new cytogenetic abnormality probably suggests the need for additional therapy. Anthracycline- or amsacrine-based combinations probably are reasonable therapeutic options, but other combination chemotherapy could be equally acceptable. As in the case of blast crisis occurring primarily in the bone marrow, the occurrence of these phenomenan in extramedullary sites in young people with HLA-compatible siblings is a specific indication for performing a bone marrow transplant.

Prevention of Blast Crisis

Since patients with CML can live for an indefinite period as long as the disease does not evolve into the acute phase, a reasonable approach at therapy is to prevent blast crisis while simultaneously controlling the blood counts with the simple measures noted previously. Two main efforts have been directed toward accomplishing this goal. The first employs immunotherapy, and the second involves close monitoring of the patient for signs of transformation with early initiation of intensive chemotherapy. Sokal et al. treated 48 patients with well-controlled chronic phase CML with intradermal injections of allogeneic blast cells supplemented in some instances by bacillus Calmette-Guerin.[81] It was hoped that the intradermal injection of allogeneic cells obtained from long-term cultures of blastic CML would be sufficiently immunogenic to induce a cell-mediated immune response against antigens characteristic of blastic clones. The survival advantage for the entire series of patients treated in this manner was less than 1 year and of borderline statistical significance when compared with historical controls. Since this study group was not randomized against a control arm, and since several dose schedules of immunization were employed, it is impossible to evaluate the significance of this finding. For the moment, this approach cannot be recommended on a routine basis.

An alternative to the immunotherapy described above has been the efforts at close monitoring of patients for preclinical evidence of transformation. Moore demonstrated that the *in vitro* growth pattern in soft agar of chronic phase CML is quite distinct from that of acute transformation.[57] Furthermore, the change from chronic growth pattern to acute growth pattern may precede the emergence of blastic crisis by as long as 6 months. Moore proposed that the change in growth pattern could be used to define an appropriate time for initiation of intensive chemotherapy. Early intensive treatment could prevent full evolution of the blastic phase. More recent studies by Salatan et al. in collaboration with Moore have failed to confirm this initial observation.[71] The latter study has shown that the acute leukemic growth pattern may appear and then disappear spontaneously without specific treatment. Furthermore, blastic transformation did not necessarily follow the appearance of the acute leukemic growth pattern.

An alternative to this methodology is close monitoring of the bone marrow for cytogenetic instability with initiation of intensive treatment at first appearance of cytogenetic progression. Brodsky et al. have shown that it is possible to eliminate evidence of clonal evolution once it has occurred.[7] They carefully monitored the bone marrow cytogenetics of 23 patients after early splenectomy. In this series 16 patients received aggressive treatment with busulfan, cytosine arabinoside, and 6-thioguanine at the first appearance of cytogenetic progression. In all patients it was possible to show a significant decrease in the number of aneuploid metaphases seen. As was the case with the immunotherapy trial described above, the survival was

compared only with historical controls. In this series the median survival of 72 months compared very favorably with the historical survival, which was only approximately 40 months. Whether the same type of finding would have resulted from a well-controlled trial remains to be seen, but Brodsky et al. have shown that eradication of clones with the characteristic cytogenetic findings of transformation can be eliminated. Our own unpublished observations in a limited number of patients indicate that long-term eradication of abnormal clones is possible.

Although these efforts intuitively are appealing, the trials to date have been very limited and not very well controlled, and it is therefore difficult at the present time to recommend either immunotherapy or monitoring of soft agar growth patterns of bone marrow cells. However, cytogenetic progression is an ominous finding. If a bone marrow transplant is possible, and if the patient is young and has an HLA-compatible sibling, this approach would be favored. For a patient who is not young or who does not have an HLA-compatible sibling, aggressive chemotherapy with anthracyclines in combination, amsacrine in combination, or cytosine arabinoside plus 6-thioguanine may restore the disease to the chronic phase and occasionally afford a survival advantage.

Since an enlarged spleen is the most common physical finding of CML and potentially a site of blastic transformation, the question has been asked whether splenectomy may affect the outcome of CML. Several groups have now examined this question in a large number of patients. In each series, there was no difference in survival between the splenectomized and nonsplenectomized groups. Splenectomy cannot be recommended as a standard procedure for all patients at the time of diagnosis.[43]

TABLE 5–8 Unfavorable Prognostic Features in CML

1. Splenomegaly >15 cm below the costal margin (D)[a]
2. Hepatomegaly >6 cm below the costal margin (P)[b]
3. Adenopathy (D)
4. Platelet count $<150 \times 10^8$/dl $>500 \times 10^8$/dl (D)
5. Leukocyte count $>100 \times 10^8$/dl (P)
6. Anemia (hemoglobin <10 g/dl) (P)
7. Peripheral blood blasts >3% (D)
8. Basophilia (P)
9. Bone marrow blasts >3% (D)
10. Myelofibrosis (D)
11. Aneuploidy (D)

[a] (D): definite.
[b] (P): probable.

Conclusions

In spite of our improved understanding of the nature of CML and its evolution, the disease remains fatal in most instances. Chronic phase CML is readily controlled, but thus far blastic transformation cannot be prevented and usually is associated with limited survival. It is true that a select group of younger patients who have an HLA-compatible sibling can now be cured with bone marrow transplantation, but for the majority of patients, cure of the disease is not within reach. These patients require the development of new therapies, but progress has been hampered by the benign-appearing nature of the disease. In order to facilitate this process, a number of centers have now begun to review their experience with CML with the hope of establishing a staging system.[32,87] Although many of the important prognostic features associated with more limited survival are well known (Table 5–8),[32,74,86,87] an organized staging system is not yet available. Recently the Italian group of Tura et al. proposed a system that segregates patients with CML into stage I, favorable prognosis (median survival 70 months); stage II, intermediate survival (median of 36 months); and stage III, median life expectancy of only 26 months.[87] Other systems currently in formulation may similarly separate patients into different prognostic categories. By excluding patients with good-prognosis CML from investigative (and potentially toxic) therapy and encouraging these approaches in patients with a more unfavorable life expectancy, rapid progress in the development of effective therapy may be possible.

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CHAPTER 6

Burkitt's Lymphoma: Diagnostic and Taxonomic Aspects

Gorm Pallesen

It has been 25 years since D. P. Burkitt described the African tumor which later was recognized as a malignant lymphoma and came to bear his name.[6] The establishment of an association between Burkitt's lymphoma (BL) and Epstein-Barr virus (EBV) and the identification of rare lymphomas with an identical morphology in most other countries evoked a world-wide interest out of proportion with the incidence of the disease. In 1969 a World Health Organization (WHO) memorandum specified the precise morphologic criteria for BL.[2] However, a moderate morphologic variation was allowed and interpreted as either biologic variation or as a limited degree of differentiation, and the designation "malignant lymphoma, undifferentiated, Burkitt's type" was advocated. It was later realized that most cases of BL actually do show signs of differentiation, i.e., synthesis of immunoglobulin.

The concepts of malignant non-Hodgkin's lymphomas generated by new immunologic techniques in the 1970s were reflected by a number of new classifications. Two of these, the Kiel classification[13,20] and the Lukes-Collins classification,[21] were both based on the combined use of morphologic and immunologic knowledge and have since met with widespread approval. In the Kiel classification, as in most other classifications, BL is recognized as an entity, a separate type of lymphoblastic lymphoma; whereas the position of BL is less obtrusive in the Lukes-Collins classification, where it is merely considered part of the small, noncleaved follicular center cell lymphomas. No matter what classification used, a number of recent publications concerning non-Hodgkin's lymphomas show that the morphologic demarcation of BL is still undertaken variously. This may naturally cause serious problems in other areas of scientific investigation (viral, chromosomal, and membrane marker studies) when they are based on a primary pathologic–anatomic classification.

In this chapter the morphologic, cytochemical, and immunologic features of BL that are pertinent to the pathologist in the differential diagnosis of tumors of the hematopoietic system are reviewed, and these features are discussed in relation to the taxonomic position of BL. The designations of the Kiel classification are used here; results obtained from fresh tumor biopsy material are preferred to results obtained from studies of cell lines derived from Burkitt's tumors.

Morphology

Cytology

The characteristic cytology of BL (Figure 6–1) makes it possible to decide on a firm diagnosis of BL immediately after receiving and staining a representative imprint from a surgical biopsy, a fine-needle aspiration biopsy, or a bone marrow smear. If the quality of the specimen is good, one should not delay diagnosis and treatment by waiting for the histologic section because all the characteristic features of BL are present in the cytologic specimen.

The cytologic picture shows strikingly uniform lymphoblasts with only some variation in cell size (8–25 μm) and in the amount of cytoplasm, which is usually moderate. All tumor cells exhibit the same degree of deep basophilia, whereas in centroblastic and immunoblastic lymphomas the cyto-

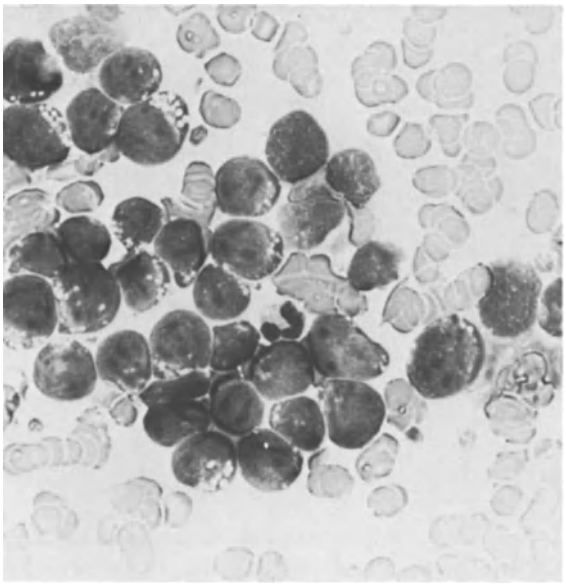

FIGURE 6–1 Cytology of BL: bone marrow smear from 16-year-old male with bulky abdominal disease. Note the number and size of nucleoli and the variation in cell size. May–Grünwald–Giemsa stain, ×720.

plasmic basophilia is variable from blast to blast. A pronounced vacuolization is usually present in the cytoplasm of BL cells, but is not a specific feature and is commonly seen in other lymphomas of high-grade malignancy (particularly the B lymphomas), nonlymphoid leukemias, and as a very distinctive feature of tumor cells in malignant histiocytosis. In BL cells a pale-staining paranuclear zone may be quite pronounced, and when the nucleus is also in an eccentric position, the BL cells may approach the plasmablast morphology.

Nuclei are regular, rounded or oval, and sometimes slightly notched. They have a rather coarse but evenly distributed chromatin. The number of nucleoli is 2–5 in most tumor cells, the size is medium, and the distribution in the nucleus is random. The nucleoli are more easily evaluated in periodic acid–Schiff (PAS) than in May–Grünwald–Giemsa stained cytologic specimens. The average size of nuclei in BL cells is about the size of the nuclei in the reactive phagocytosing macrophages, the "starry-sky" histiocytes.

Histology

The effects of insufficient fixation account for most difficulties in discriminating between BL and other lymphomas of high-grade malignancy in histologic sections. When the histologic technique is good, a safe diagnosis of BL can be made without the use of additional imprints. The salient histologic features are (a) an extreme monomorphism of the tumor cells, (b) a pronounced starry-sky pattern (Figure 6–2), with histiocytes often being loaded with tumor cells or their fragments, and (c) cohesiveness of tumor cells in well-fixed areas.[2] The starry-sky phenomenon is often present in other lymphomas of high-grade malignancy and in myeloid sarcoma, and is by no means diagnostic of BL. However, it is usually most pronounced in BL, and besides it serves as a very useful internal control comparing the lymphoblastic and histiocytic nuclear sizes.

The term "cohesiveness" is unfortunate as it points to features of epithelial tumors that form intercellular connections. The term "syncytial" growth, as used in diagnostic cytology, would seem more appropriate, meaning the formation of apparently syncytial clusters of tumor cells with cell borders being indistinct or invisible at the light microscopic level. This tendency of BL cells to form syncytial clusters is shared with cells of other B lymphomas of high-grade malignancy and may even occur in myeloid sarcoma but is not seen in lymphoblastic lymphomas of convoluted cell type or non-B–non-T type.

In formalin-fixed histologic material the BL cells reveal a prominent nuclear membrane and

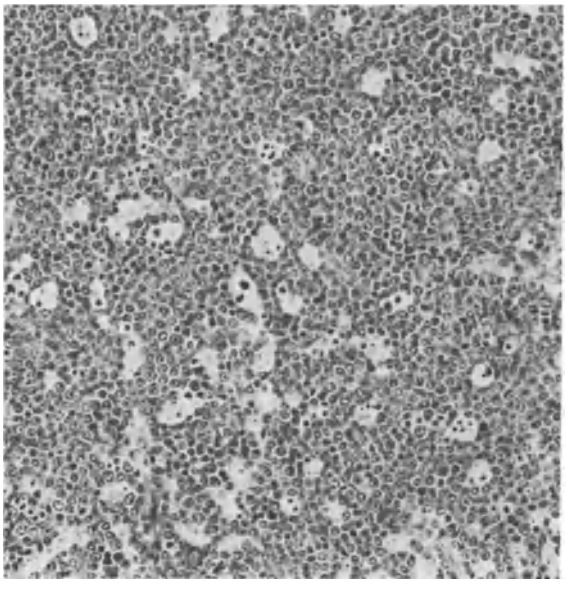

FIGURE 6–2 BL with prominent starry-sky pattern in bilateral ovarian tumor. The 38-year-old patient also had involvement of both mammary glands and peripheral lymph nodes. Giemsa stain, ×180.

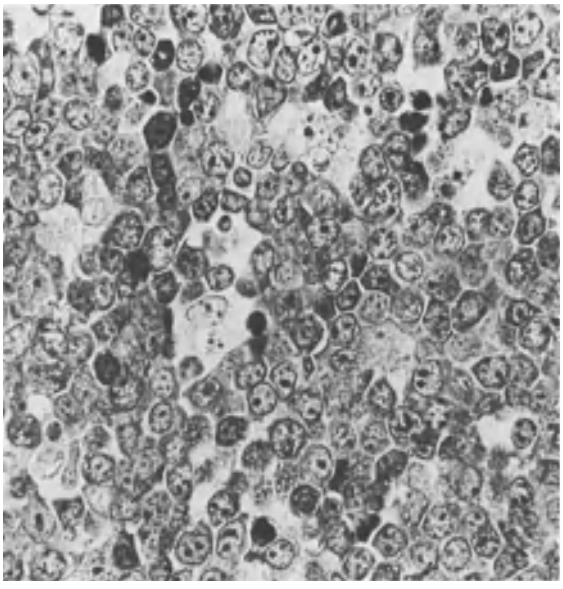

FIGURE 6–3 Section of lymph node with BL from 9-year-old boy seen at high magnification. Note that lymphoblast nuclei are about the size of histiocyte nuclei. Nucleoli are medium sized and randomly distributed. Giemsa stain, ×720.

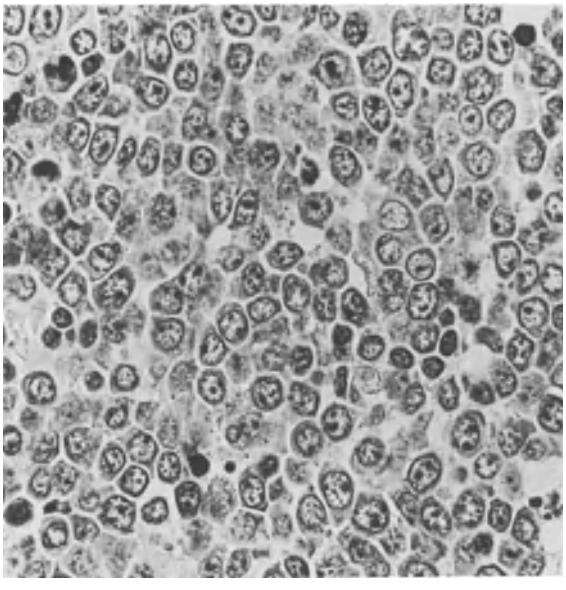

FIGURE 6–4 Section of lymph node from 6-year-old boy. Note the extreme monomorphism of the lymphoblasts, which fulfill the cytologic criteria of BL cells. The patient had involvement of the abdomen and bone marrow. The tumor was Epstein-Barr virus negative. The tumor cells expressed Fc-IgG receptors and surface and cytoplasmic immunoglobulin (IgM kappa). In cryostat sections, HLA-DR and CALLA were demonstrated on the tumor cell surfaces using immunoperoxidase staining with monoclonal antibodies. Giemsa stain, ×720.

a coarse chromatin with an irregular distribution in a relatively clear parachromatin. The nuclear chromatin in BL is more coarse than in centroblastic and immunoblastic lymphomas.

When these histologic features (Figures 6–2, 6–3, and 6–4) are combined with the cytology described above, the pathologist should diagnose BL irrespective of clinical data such as the age of the patient and tumor localization. Probably the most outstanding feature of BL is the extreme monomorphism of neoplastic cells that look as if they all were "frozen" at a similar stage of cellular differentiation. However, deviation from the prototypic BL occurs. Cellular pleomorphism with bi- or multinucleate BL cells may be seen in relation to necrosis, e.g., in large ovarian tumors (Figure 6–5), in tumors influenced by chemotherapy,[28] and in recurrent tumors; but the majority of the tumor cells usually exhibit a typical cytology even in such cases.

A very interesting histologic feature of BL was described by Mann et al.,[24] who found germinal centers focally involved in lymph nodes and Peyer's patches in 10 biopsies and 2 autopsies from American patients with BL. The authors concluded that BL cells are related to B lymphocytes of normal germinal centers, and may either arise

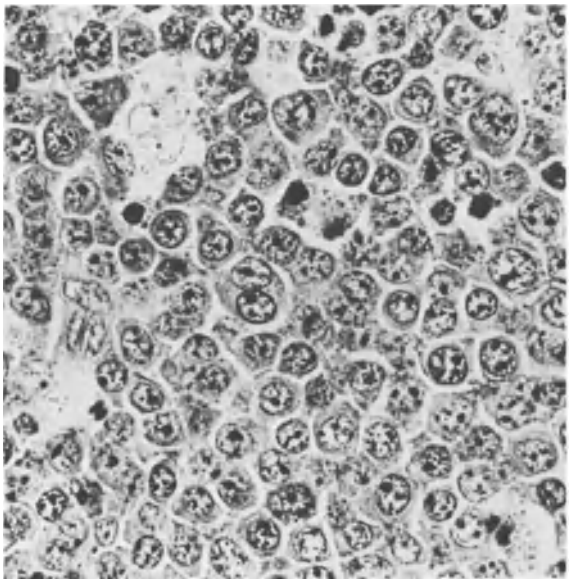

FIGURE 6–5 BL with cellular pleomorphism. Slight increase in cell size and binucleation of some tumor cells occurred in huge bilateral ovarian tumors with necrosis in a 16-year-old patient. Immunoperoxidase staining of fixed tissue revealed weak expression of cytoplasmic IgM lambda. Giemsa stain, ×720.

from germinal center cells or follow migration pathways resulting in the selective "homing" to germinal centers.

We recently confirmed and extended the findings of Mann et al.[18] In a retrospective study of Danish patients with BL, we had the opportunity to study the very early infiltration with BL cells in lymph nodes from two boys. A single lymph node from the neck was studied in one of the patients, who had bulky abdominal and paravertebral disease and was leukemic—French–American–British (FAB) type L_3—at presentation. In the other patient we noticed the homing phenomenon in several mesenteric lymph nodes regional to an ileocoecal Burkitt's tumor. The infiltration was discrete and had passed unnoticed at the primary microscopy. In addition to the germinal center involvement previously described by Mann et al., we noticed that this stage was preceded by a particular stage in which the lymphocytic mantle zone was replaced by BL cells, with germinal centers remaining intact (Figure 6–6). Subsequently the whole germinal center was replaced by neoplastic cells (Figure 6–7), and finally these foci enlarged and fused into an entirely diffuse pattern.

An organoid, follicular growth pattern as seen in centroblastic and in centroblastic/centrocytic lymphomas is not a feature of BL, and the homing phenomenon looks quite different from that. Our observation of mantle zone homing prior to germinal center involvement in BL may indicate that BL cells are not derived from the cellular constituents of normal germinal centers but rather from a pre– or post–germinal center cell type.

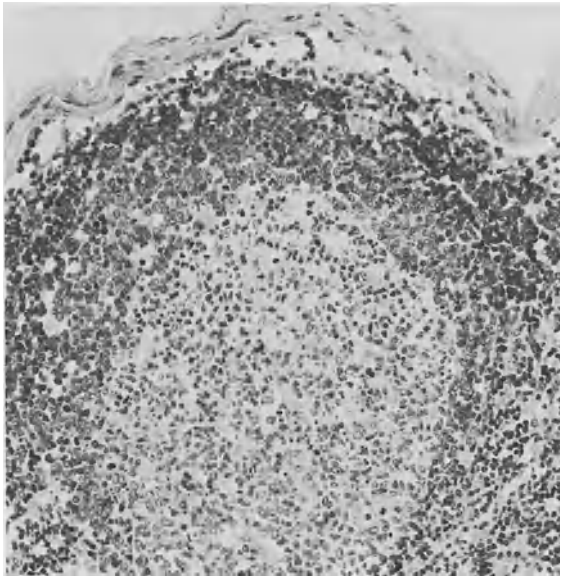

FIGURE 6–6 BL with "homing" phenomenon: early lymph node involvement, with the lymphocytic mantle zone replaced by neoplastic cells. The germinal center is intact. Same patient as in Figure 6–3. Giemsa stain, ×180.

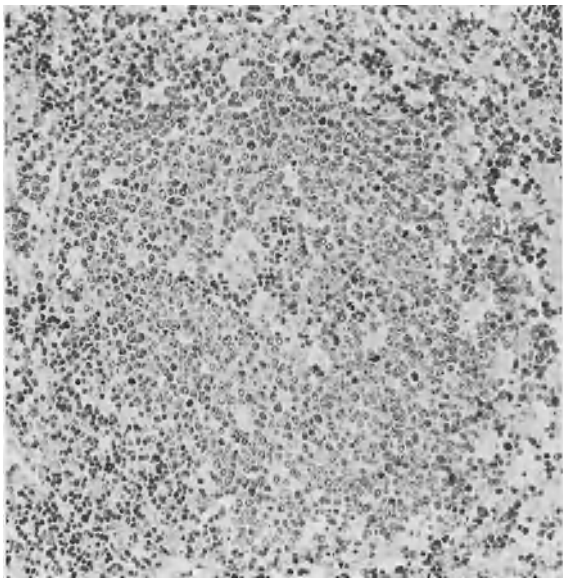

FIGURE 6–7 BL with "homing" phenomenon: lymphocytic mantle zone and germinal center replaced by tumor cells (except for a few centrocytes centrally). BL cells are also seen traveling in the sinuses in the interfollicular area. Same lymph node as in Figure 6–6. Giemsa stain, ×180.

Ultrastructure

The light microscopic features of BL naturally may also be identified at the electron microscopic level. The main additional information provided by electron microscopy is that (a) a large number of polyribosomes occur in the cytoplasm, (b) rough endoplasmic reticulum is scarce, and (c) there is a general scarcity of other cytoplasmic organelles.[2] These structural features correspond to (a) the intense cytoplasmic basophilia obtained with the Giemsa stain, (b) the low content of cytoplasmic immunoglobulin, and (c) the weak or absent reaction for most enzyme stains.

It is my opinion that electron microscopy is of little importance in the diagnostic process of non-Hodgkin's lymphomas. The subclassification can be made quickly, cheaply, and accurately if the histologic technique is of a sufficient quality and if it is combined with the routine use of cyto-

logic, cytochemical, and sometimes immunohistochemical examinations performed on imprints and sections of tumor tissue.

Cytochemistry

The cytochemical features of nonendemic BL[10,24] (personal observations) are identical with those of African BL previously studied by Wright.[42] The cytoplasmic vacuoles contain neutral fat and stain with Sudan black and oil red 0. This is not a specific feature of BL, since fat vacuoles occur commonly in other high-grade malignant lymphomas, (i.e., centroblastic and immunoblastic).

The PAS reaction is usually negative, however, glycogen granules may be stained in occasional tumor cells. Coarse glycogen granules are typical of lymphoblasts in lymphoblastic lymphomas other than BL. One reported case of American BL showed a markedly increased PAS reaction, however that case also deviated in other respects from typical BL and might belong to another lymphoma subclass.[34] Tartrate-sensitive acid phosphatase is usually present in BL cells and shows a rather weak, dispersed granular pattern, whereas nonspecific esterase is weak or absent.

Alkaline phosphatase was not reported in BL cells by Wright[42] or by Berard et al.[2] whereas Mann et al.[24] found partial expression of this enzyme in one of eight cases examined. In five of my own cases (unpublished results) alkaline phosphatase was absent. The BL cells were consistently devoid of peroxidase, naphthol-AS-D-chloroacetate esterase, lysozyme, α_1-antitrypsin and α_1-antichymotrypsin. The presence of these marker enzymes in hematopoietic tumor cells is indicative of a myeloid or a monohistiocytic derivation.

Immune Markers

Expression of Immunoglobulin

Surface Immunoglobulin

The presence of surface membrane immunoglobulin (SmIg) was reported as early as 1967 on tumor cells of biopsy specimens and on derived cell lines from a case of endemic BL.[19] The results of SmIg studies in many of the early works on African BL are difficult to interpret because the techniques to detect SmIg were less developed than today, or because the studies were performed on cell lines and not on tumor specimens.[11,39] In cultured cell lines established from endemic BL, Takahashi et al. found an extreme imbalance of heavy and light chain expression, however, IgM was the most frequent heavy chain encountered.[39]

More recently, Gunvén et al. reported their findings of SmIg in cell suspensions of 91 cases of African BL.[16] Only 5% of their cases did not express SmIg, and the majority expressed IgM. A few cases expressed simultaneously mu and gamma chains with a single light chain, and delta chains were partially expressed in 2 of 53 biopsies examined. Magrath et al.,[23] in a recent study of surface markers in lymphoma-derived cell lines, made the interesting observation that SmIg was expressed strongly in all 7 EBV nuclear antigen–negative cell lines studied, while SmIg was weak (6 cases) or absent (4 cases) in cell lines positive for EBV nuclear antigen.

In the rare cases of nonendemic BL, a pattern of SmIg expression emerges through the many recent reports. In an ongoing multiple cell marker study of non-Hodgkin's lymphomas, we have encountered 3 cases of nonendemic BL among more than 50 high-grade malignant lymphomas examined during a 3-year period (G. Pallesen, M. Madsen: unpublished results). One of our cases did not express immunoglobulin, but the others expressed SmIg strongly, i.e., IgM kappa and IgGM kappa.

If our cases are included with published cases, SmIg has been analyzed in at least 85 cases of nonendemic BL;[1,3-5,7,9,12,15,17,24,26,29,31,32,35,37,41] 27 of these cases[31,32,35] were designated Burkitt's cell leukemias (FAB type L_3). Whereas most cases were stated to express SmIg strongly, 4 cases were found to be nonexpressive (our unpublished case).[4,26,31] Details of immunoglobulin chain typing were not presented in all publications, however mu chains were reported in 56 cases: in 2 of these, with the simultaneous expression of gamma chains and a single light chain (our unpublished case);[17] and in 7 of these, in association with delta chains.[1,4,24,31] Gamma chains alone were found in 4 cases[26,31] and alpha chains in one case.[31] Light chains alone were reported in 1 case.[35] The distribution of light chains (29 cases with kappa, 25 cases with lambda) is imbalanced in favor of lambda, as previously pointed out by Preud'homme et al.[31] and by Kerndrup and Pallesen.[18]

The real frequency of delta chain expression in nonendemic BL is unknown because examination for this differentiation marker was not in-

cluded in most studies. We did not find delta chains in our 3 cases, while Preud'homme et al.[31] found delta associated with mu chains in 3 of 10 cases (BL cell leukemias) studied.

Cytoplasmic Immunoglobulin

Little is known about the expression of cytoplasmic immunoglobulin (CIg) in endemic and nonendemic BL, although this character is important in the taxonomic evaluation of differentiation in malignant B lymphomas. In a recent paper Wright maintained that CIg is never seen in BL, as opposed to follicular center cell lymphomas.[43] This statement is not correct in the case of nonendemic BL, and probably not even in African BL, because Minowada et al.[25] have already demonstrated CIg in a cell line derived from BL. However, Wright has studied about 20 African tumors with an immunoperoxidase technique and was not able to detect CIg in any case (personal communication).

In nonendemic BL only few studies have included investigation for CIg. Pallesen et al.[27] demonstrated weak reactions for CIg in 2 of 2 Danish cases using an immunoperoxidase method, and Habeshaw et al.[17] found CIg in 2 of 3 English cases. In a later study we were able to detect CIg in 7 of 8 Danish BL cases, although reactions in general were weak.[18] For comparison, we found monotypic CIg in 3 of 6 centroblastic lymphomas, which all expressed SmIg, whereas 13 of 14 immunoblastic B lymphomas expressed CIg (G. Pallesen, M. Madsen: unpublished results).

Expression of Receptors for Fc-IgG and C3

No clear data are available about the expression of receptors for IgG in fresh biopsies from African BL, whereas C3 receptors were partially expressed by BL cells from six tumors examined by Magrath.[22] Various cell lines derived from endemic tumors have been shown either to express or lack these receptors.

Tumor cells from nonendemic BL have been examined for receptors in a number of cases. Among the 3 cases in our own study (all were EBV nuclear antigen negative), we found partial expression of C3 receptors in a case that did not express SmIg or CIg. Receptors for IgG were partially expressed by tumor cells in another case, while the third case was devoid of both receptor types (G. Pallesen, M. Madsen: unpublished results). When our cases are combined with those in the literature, the following figures result: receptors for IgG were found in 23 of 49 cases of nonendemic BL,[3,4,17,24,31,32,35,37] whereas C3 receptors were expressed by 8 of 24 cases.[3,15,17,24,32,36,37] In the individual BL case tumor cells are apparently heterogenous in their ability to express these receptors. In our cases, as well as in those of Mann et al.,[24] they were usually expressed by a minority of the tumor cells only.

Expression of Other Cell Markers

Receptors for mouse erythrocytes were absent from 13 cases examined by Bernard et al.[4] and 5 cases examined by Stein et al.[37] Receptors for Fc-IgM were demonstrated in 1 of 2 BL cases examined.[17] HLA-DR antigen has consistently been demonstrated on the surface of BL cells.[3,4,33,37]

Common acute lymphoblastic leukemia (ALL) antigen (CALLA) was recently demonstrated in 5 of 6 cases by Ritz et al.[33] using a monoclonal antibody (J5). CALLA, which used to be considered a marker of early hematopoietic stem cells, was also demonstrated in lymphoblastic lymphomas of T cell type and in differentiated B cell lymphomas such as the centroblastic/centrocytic. CALLA was not demonstrated in 14 BL cases examined by Bernard et al.[4] In 2 of our BL cases, in which frozen-stored tissue was available, we were able to detect CALLA using J5 and an immunoperoxidase technique on cryostat sections. Terminal deoxynucleotidyl transferase, a marker of T cell ALL and common ALL but not of B cell ALL, was found in 2 of 14 BL cases studied by Bernard et al.[4]

Recently Wiels et al. reported on the generation of an interesting monoclonal antibody referred to as 38.13, which recognized an antigen associated with BL.[40] The antigen was expressed by most EBV-positive and -negative BL cell lines and on cells from both endemic and nonendemic cases. It was not expressed by normal cells from blood, bone marrow, and normal lymphoid tissues, nor by various hematopoietic neoplasias.

Differential Diagnosis

The pertinent disorders to be considered in the differential diagnosis of BL comprise nonlymphoid hematopoietic neoplasias, such as malignant his-

tiocytosis and myeloid sarcoma, and the various lymphocyte-derived neoplasias of high-grade malignancy, such as the centroblastic, immunoblastic, and lymphoblastic lymphomas.

Malignant Histiocytosis

In malignant histiocytosis the tumor cells are large and usually pleomorphic with abundant cytoplasm in which erythrocytes may be present due to phagocytosis. If imprints are obtained, stainings for nonspecific esterase and acid phosphatase reveal strong diffuse reactions that are never seen in lymphoid cells, however, they may also be seen in carcinomas and in malignant melanoma. In the formalin-fixed and paraffin-embedded tissue, α_1-antitrypsin and α_1-antichymotrypsin can be demonstrated immunohistochemically in the cytoplasm of malignant histiocytes, whereas lysozyme (muramidase) is only expressed occasionally.

Myeloid Sarcoma

Immature myeloid sarcoma must be considered in the differential diagnosis because a starry-sky pattern as well as a cohesive/syncytial growth pattern may be present. The following case report is illustrative of the diagnostic difficulties in a case of myeloid sarcoma:

A 9-year-old girl was referred to us with a diagnosis of BL. She had a large tumor involving the maxilla and orbita, and a previous biopsy revealed a focally pronounced starry-sky pattern and syncytial groupings of tumor cells. In a subsequent fine-needle aspiration biopsy, we rejected the possibility of BL because of a cytology incompatible with this diagnosis. After prolonged search we found a few cells with fine myeloid granulation of the cytoplasm and diagnosed a myeloid sarcoma of a very immature type (FAB type $M_{1/2}$).

The diagnosis was verified when an involved lymph node was removed (Figure 6–8). In imprints from this a few blasts reacted positively with the Sudan B or peroxidase, and the chloroacetate esterase stains, and in tissue sections lysozyme could be demonstrated in a few blasts using an immunoperoxidase method, whereas the chloroacetate esterase stain of sections was negative. A few weeks later she developed an acute myeloid leukemia with distinctive differentiation (FAB type M_2) of blasts in blood and bone marrow.

The differentiated myeloid or granulocytic sarcomas may be diagnosed by conventional histology if the technique is good. If less than optimal, staining of tissue sections for chloroacetate esterase or lysozyme, or processing of tissue for electron microscopy may be necessary to obtain the correct diagnosis.

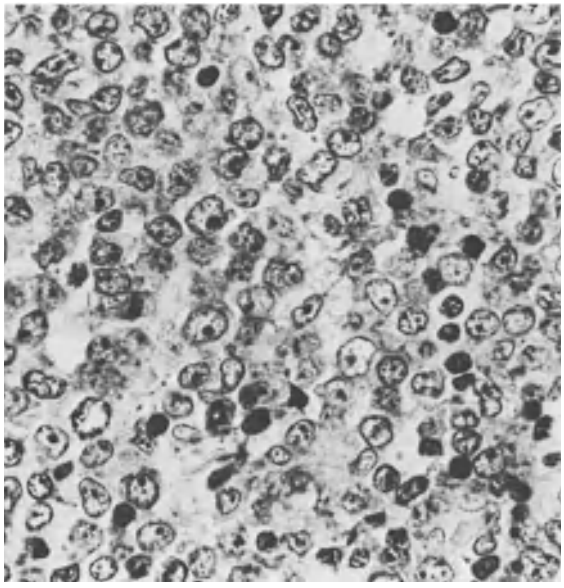

FIGURE 6–8 Very immature myeloid sarcoma. The tumor presented in the maxilla and orbita in a 9-year-old girl. Due to clinical features and prominent starry-sky pattern in her first biopsy she was referred with a diagnosis of BL. The microphotograph is from a subsequent biopsy of a cervical lymph node. The cytologic criteria of BL are not fulfilled. Monomorphism is absent and the blasts are of varying size and usually too large. Nuclei are irregular with few but large nucleoli. Giemsa stain, ×720.

Centroblastic Lymphoma

The centroblastic lymphoma is morphologically and presumably also taxonomically close to BL. In well-fixed tissues the centroblasts reveal 1–4 nucleoli, which are medium sized and often associated with the nuclear membrane; in contrast to BL cells, the chromatin is finely dispersed (Figure 6–9). The cytoplasm is scant to moderate, variably basophilic, and sometimes contains fat vacuoles. The centroblastic lymphoma may be diffuse or nodular. In the latter case the proliferation is usually associated with many centrocytes.

Immunoblastic Lymphoma

The designation "immunoblastic lymphoma" is widely used in modern pathology and is attached to lymphomas with a predominance of tumor cells of the transformed lymphocyte type, i.e., large blastoid cells with ample cytoplasm and nuclei with delicate chromatin and 1–2 large nucleoli cen-

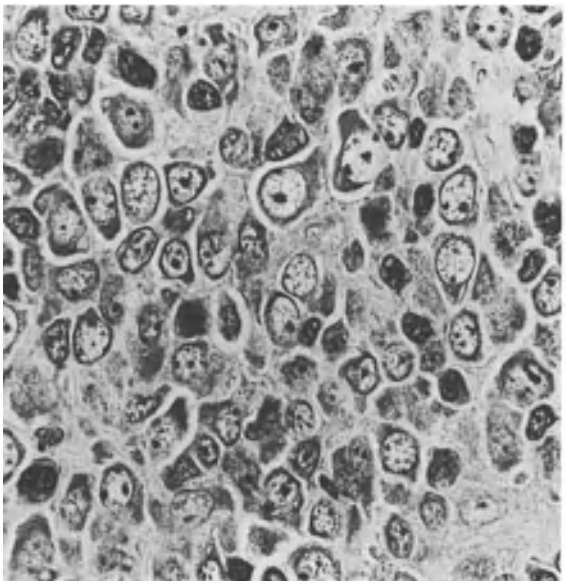

FIGURE 6–9 Malignant lymphoma, centroblastic (nodular type). Characteristic centroblasts predominate in this lymph node from a 53-year-old woman. The cells are large; nuclei are vesicular with delicate chromatin and 2–3 medium-sized nucleoli, which are often associated with the nuclear membrane. Cytoplasmic basophilia varies. Centrocytes occur intermingled with small and large centroblasts. Monotypic IgM lambda was expressed strongly as both surface and cytoplasmic immunoglobulins. Giemsa stain, ×720.

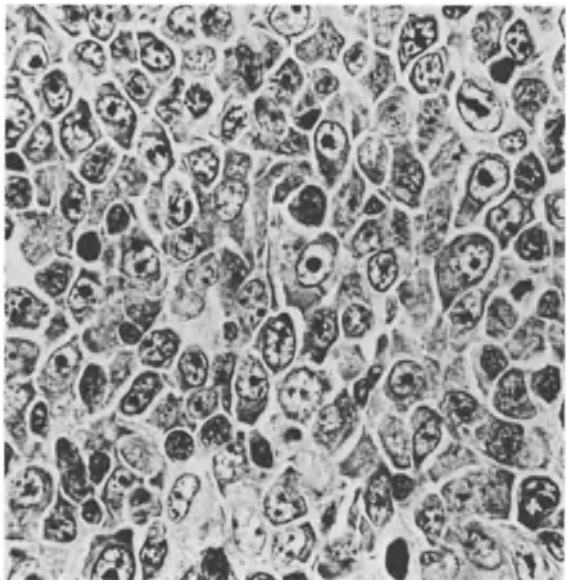

FIGURE 6–10 Malignant lymphoma, B immunoblastic. Typical immunoblasts with large central nucleolus prevail, however, scattered plasmablasts occur in this lymph node from a 72-year-old male. Note that cells are large with abundant cytoplasm with varying basophilia. There is a moderate pleomorphism. Monotypic immunoglobulin (IgM lambda) was demonstrated in the cytoplasm as well as on the cell membranes. Giemsa stain, ×720.

trally located (Figures 6–10 and 6–11). By means of immunologic methods, immunoblastic lymphomas with almost identical morphology may be further divided into phenotypes of the B or T lymphocyte lineage, or they may be nonexpressive (0 types). In a study of 53 lymphomas of high-grade malignancy, we classified 19 cases as immunoblastic lymphomas according to histology. As many as 15 were B derived, only 2 were T derived, and 2 were nonexpressive (G. Pallesen, M. Madsen: unpublished results). It was remarkable that SmIg and CIg were expressed simultaneously and usually strongly in 12 of 14 cases examined for both.

A clue as to the immunologic type of immunoblastic lymphomas is given when some immunoblasts show plasmablastic (B cell) differentiation (Figure 6–10), whereas T immunoblastic lymphoma (Figure 6–11) is suggested if nuclear contours are complex and irregular, or if nuclei are elongated. In our T immunoblastic lymphomas a broader spectrum of cell types was seen than in the B types. Apart from the characteristic immunoblasts, smaller blasts and large and intermediate-sized lymphocytes were components of T immunoblastic lymphomas. Other histologic features in favor of the T immunoblastic lymphoma comprise selective involvement of the T zone, proliferation of postcapillary venoles, and increased numbers of histiocytes and eosinophils.

Lymphoblastic Lymphomas Other than Burkitt's Lymphoma

The lymphoblastic lymphomas other than BL comprise, according to the Kiel classification, B lymphoblastic lymphomas other than BL, T lymphoblastic lymphomas with and without convoluted nuclei, and unclassified, which includes the lymphoma counterpart to common ALL.

The B lymphoblastic lymphomas other than BL exhibit a cytology similar to BL but lack a starry-sky picture and typical "cohesiveness" of the tumor cells.[20] However, histologic criteria sufficient for a clear distinction between a Burkitt's type and non-Burkitt's type B lymphoblastic lymphoma

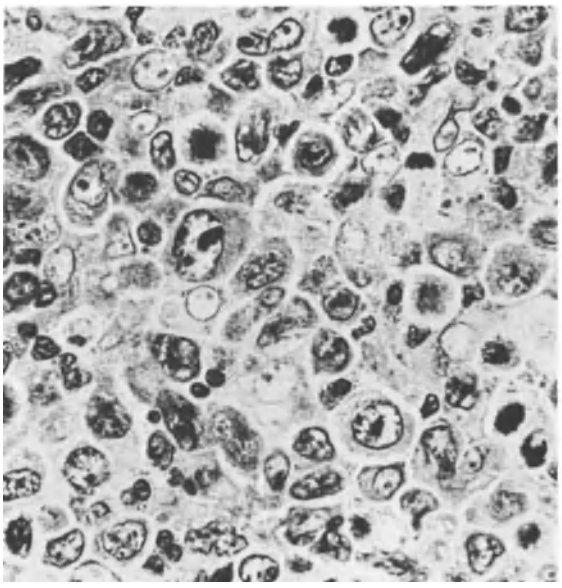

FIGURE 6–11 Malignant lymphoma, T immunoblastic. A much wider variation of immunoblasts is seen in T than in B immunoblastic lymphomas. In this lymph node from a 61-year-old male, the T immunoblasts are intermingled with smaller blasts and large T lymphocytes, and most of these reveal a very complex nuclear structure (which is typical of most peripheral T lymphomas). Many eosinophils participate in the proliferation. Tumor cells formed rosettes with sheep erythrocytes. Immunoperoxidase staining of cryostat sections with monoclonal antibodies revealed the membrane phenotype of T helper lymphocytes: OKT3+, OKT4+, OKT6−, OKT8−, Lyt-2+, Lyt-3+, HLA-DR− and Ig−. Giemsa stain, × 720.

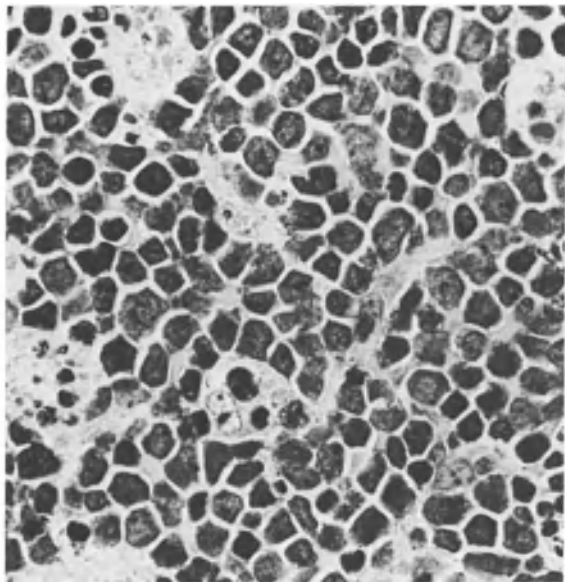

FIGURE 6–12 Malignant lymphoma, T lymphoblastic with convoluted nuclei. Cervical lymph node from a 14-year-old boy with mediastinal tumor shows a starry-sky pattern. Note the small size and barely visible cytoplasm of most lymphoblasts. Nuclei are often convoluted, chromatin is condensed but evenly distributed, and nucleoli are inconspicuous. Immunoperoxidase staining of cryostat sections in this case revealed the membrane phenotype OKT3+, OKT4+, OKT6+, OKT8−, Lyt-2(+), Lyt-3+, HLA-DR−. Giemsa stain, × 720.

are hardly available at present. A tumorous form of pre–B cell ALL, a pre–B lymphoma, has not yet been identified.

The T lymphoblastic lymphomas of convoluted cell type are composed of blasts that are smaller than BL cells. Their nuclear chromatin is condensed and nucleoli are inconspicuous (Figure 6–12). A starry-sky pattern is sometimes present but not as prominent as in BL. The feature of nuclear convolution is characteristic but may be seen also in cases of nonexpressive lymphoblastic lymphomas. A strong focal acid phosphatase reaction is very specific and allows a clear distinction of this lymphoma from other lymphoblastic neoplasias. Most cases express receptors for sheep erythrocytes or T-associated surface antigens.

T lymphoblastic lymphomas of nonconvoluted cell type occur rarely. I have seen only one proven case, arising in a 4-year-old boy without mediastinal tumor. The histology of this case was quite characteristic (Figure 6–13) and much different from that of the convoluted cell type. The relatively uniform population of blasts were reminiscent of immunoblasts, however, they were smaller. As in immunoblastic lymphoma, a single and rather prominent nucleolus was seen. It seems that the acute leukemia counterpart to this lymphoma would be designated L_2 according to the FAB classification. Cytochemically, the blasts were devoid of acid phosphatase, but a strong dotlike reaction was seen with the stain for acid nonspecific esterase, as in normal peripheral T helper lymphocytes. The surface markers were revealed by immunostaining frozen tissue sections with monoclonal antibodies using an immunoperoxidase method. The blasts were OKT3−, OKT4+, OKT6−, OKT8−, Lyt-2+, Lyt-3+, HLe-1+, HLA-DR+, SmIg− and CIg−. This phenotype probably indicates a postthymic T cell origin or differentiation, whereas our cases of convoluted cell type all expressed thymus cortex antigen (OKT6+).

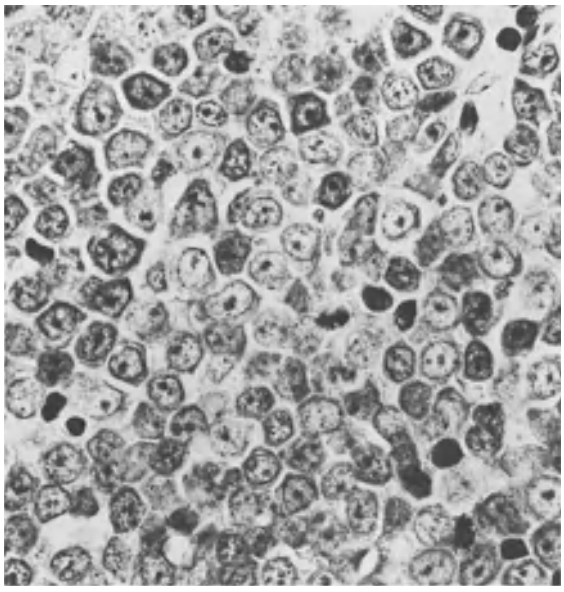

FIGURE 6–13 Malignant lymphoma, T lymphoblastic without convoluted nuclei. Cervical lymph node from a 4-year-old boy without mediastinal tumor or leukemia. The lymphoblasts are reminiscent of small immunoblasts with one prominent nucleolus in a delicately stippled chromatin. The cytoplasm is distinct and moderate in amount. Giemsa stain, × 720.

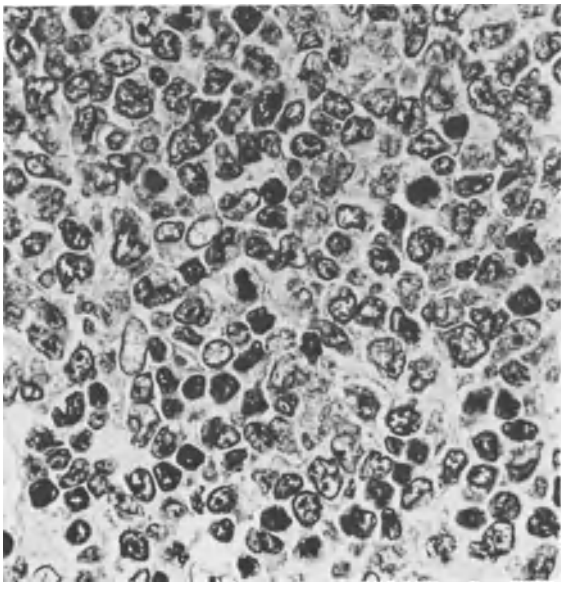

FIGURE 6–14 Malignant lymphoma, lymphoblastic, unclassified. Relatively small blasts with irregular nuclei are seen in this lymph node from a 14-year-old boy. The chromatin is stippled and the cytoplasm is indistinct. The immune phenotype of this case was non-B–non-T. Giemsa stain, × 720.

The unclassified lymphoblastic lymphomas comprise proliferations of small to medium-sized blasts cytologically different from the blasts of BL and convoluted cell type, and are immunologically devoid of markers of differentiated lymphocytes. Most cases are apparently sarcomatous counterparts to common ALL. I have studied relatively few cases; one case with small blasts and pronounced nuclear indentations is shown in Figure 6–14. Neither immunoglobulin nor receptors were expressed by the lymphoblasts, which in contrast showed coarse diastase-sensitive granulation in PAS-stained imprints. In the illustrated case (Figure 6–14), the nuclear chromatin was rather stippled, whereas in other cases it may be more evenly distributed. The main histologic difficulty in lymphoblastic lymphoma, unclassified, is to recognize this as a basically high-grade malignant proliferation of small lymphoblasts and not merely as large lymphocytes of a low-grade malignant lymphoma. Imprints are of significant value in this evaluation.

Taxonomic Problems

Although the diagnosis of BL has been based on strict morphologic criteria since 1969,[2] there is still an obvious lack of agreement among pathologists as to what constitutes BL or non-BL. Naturally, it is tumors that exhibit slight variation from the prototype (a variation which may be allowed according to the WHO criteria) that cause the problems. Some authors have represented the point of view not to allow any deviation from the typical monomorphic appearance of BL at all.[8,43] Dura et al. accepted as BL only 4 of 65 chiefly abdominal cases of American childhood B lymphoblastic lymphomas.[8] Certainly, true cases of BL might be lost using their criteria.

Most other authors have been less restrictive when applying the WHO criteria in cases of nonendemic BL.[4,7,18,20,24,28,30,31] In a study of a large French series of childhood lymphomas, Philip et al.[30] expressed the point of view opposite to Dura et al.[8] They diagnosed 57.3% as BL. They stated that the incidence of nonendemic BL had been underestimated and that BL is the most frequent childhood lymphoma in the world. The histologic criteria practiced by Philip et al. may carry the risk of including cases of non-BL among "true" cases of BL.

It is evident that lack of definition about the

extent and the type of variation allowed in BL[2] is the main responsible factor for the inhomogeneous classification. Expressions such as "atypical BL," "BL-like," or "BL type" have repeatedly been used in the literature in prudent attempts to denote cases of BL at slight variance. Like BL, the centroblastic, immunoblastic, and lymphoblastic lymphomas may show morphologic variation from their defined prototypes, rendering otherwise useful diagnostic criteria[20,21] less valuable, and thereby enhancing the taxonomic difficulties.

The main difference between tumor cells of endemic and nonendemic BL is with respect to their association with EBV; they are similar with respect to morphology, surface markers, and kinetic and cytogenetic features. There is as yet no morphologic evidence that EBV-associated and -unassociated BL cell proliferations belong to different lymphocyte subpopulations, and it should be kept in mind that morphologic variation is also met with in EBV-positive tumors. The first European BL associated with EBV, published by Goetz et al.[14] and depicted by Lennert and Mohri,[20] was composed of rather large cells exhibiting features of BL cells, sometimes of centroblasts and occasionally of immunoblasts (original sections are available in the Lymphoma Registry in Kiel). This case certainly is a candidate for the designation "Burkitt-like" only.

Accordingly, the slight morphologic variation so often reported in BL by experienced pathologists[2] could hardly altogether result from inaccuracy of examiners or from poor histologic technique. It might instead reflect a true spectrum of morphologic phenotypes in BL, although it is narrow compared with other lymphoma subclasses. The slight morphologic variation of BL cells between different tumors and within a single tumor may be a reflection of their heterogeneous functional capacities, i.e., their partial expression of Fc or complement receptors, or of SmIg chains. The opinion advanced for many years that BL cells belong to an *undifferentiated* lymphocytic cell population was based exclusively on morphology and should now be completely abandoned. The "homing" to cortical follicles (mantle zone and follicular center) may denote the only morphologic sign of some as yet not understood commitment of the BL cells.

In considering the functional capacities as revealed by immune marker techniques, it is obvious that BL cells express the markers of differentiated rather than immature lymphocytes. Surface IgD, which is found predominantly associated with IgM on the surfaces of normal mantle zone lymphocytes, is acquired and lost again during B cell maturation. The delta chain–negative stage may therefore correspond to either an immature or a mature stage of B lymphocyte differentiation. Delta chain expression in African BL was low in the study of Gunvén et al., and they concluded that it placed the BL cell at an early stage of lymphoid cell maturation.[16] Preud'homme et al. made the observation of low delta chain expression in nonendemic BL.[31] However, they favored an advanced maturational arrest because SmIg of IgG or even IgA type was expressed in some of their cases, and because a few of their patients had an increase in monoclonal serum immunoglobulin.

Tumor cells were unfortunately not investigated for CIg by any of these groups. The finding by others of CIg occurring simultaneously with SmIg in BL cells denotes an advanced stage of differentiation (late maturational arrest). Accordingly, in a taxonomic sense, BL cells are not lymphoblasts (lymphocyte precursors) but transformed lymphocytes (plasma cell precursors) different from B immunoblasts. The presence of CALLA on tumor cells of BL is no argument against this statement because this antigen is also expressed by differentiated lymphocytes of centroblastic/centrocytic lymphoma and by various unrelated tissues. Consequently, CALLA can hardly be considered a differentiation antigen any longer.[33]

The counterpart to the BL cell in normal lymphoid tissues is unknown. The early homing to the mantle zone might point to a derivation from mantle zone lymphocytes, which are characterized by marked expression of delta chains and alkaline phosphatase on their cell surfaces; however, BL cells express these markers infrequently. The rather weak or absent expression of complement receptors in BL makes the concept of a derivation from germinal center cells less attractive because these express C3 receptors strongly.

The presence or absence of EBV has become a point of taxonomic importance in the Kiel classification. EBV-positive tumors are designated "Burkitt's lymphoma," whereas EBV-negative cases with BL morphology are designated "BL type." I have not undertaken the distinction in this chapter. The term "Burkitt's type" has been used in various ways independently of the Kiel classification, and the tradition among pathologists

does not favor the implication of viral criteria in a basically morphologic classification. Moreover, inclusion of viral criteria may be unfortunate because it carries the risk of concealing the occurrence of EBV in lymphomas of different histologic type. The lymphoma described by Suzuki et al.[38] should not be referred to as BL because of the presence of the EBV genome in the tumor cells; according to morphology it was definitely a case of immunoblastic lymphoma.

Conclusions and Recommendations

The morphologic criteria of BL published in the WHO memorandum[2] are still unchallenged. The creation of new lymphoma classifications in the 1970s brought clarity to the classification of most cases of high-grade malignant lymphomas, which may now be arranged in relatively few morphologic entities. However, BL with less than perfect monomorphism may persistently cause disagreement and uncertainty among pathologists, and may constitute a permanent risk of contaminating materials. It would seem that this could partly be compensated for by exchange of material for reciprocal review.

Concerning morphology, the findings of early and selective involvement of the lymphocytic mantle zone and subsequently the germinal center should be looked for in endemic BL, in which it has not been reported. This feature may be unique to BL, and if present also in African cases, it would bring further support to the concept that the two types of BL are truly identical.

Concerning expression of cell markers, no obvious difference has been disclosed between African and nonendemic BL. However, nonendemic BL, including its leukemic form has been far more extensively studied with modern methods than has African BL, in which cell lines have been the main source of material for marker analyses.

The marker studies have shown that BL cells are not undifferentiated lymphoblasts, and consequently the designation "undifferentiated lymphoma" should be avoided. On the contrary, BL cells may be representative of the transformed stage of relatively differentiated B lymphocytes because (a) SmIg is expressed strongly in most cases, (b) immunoglobulin heavy chains other than mu are sometimes expressed, (c) delta chains are expressed infrequently, and probably the BL cell has passed the maturational stage of delta chain expression, and (d) CIg is expressed in at least some of the cases, although weakly. Other cell markers, such as receptors for Fc-IgG are expressed in almost one-half of cases, and complement receptors in about one-third of cases.

The question of CIg expression is of crucial taxonomic importance. Because of the controversy as to whether CIg is expressed in African BL or not, it would seem pertinent to investigate a larger number of both African and nonendemic BL tissues with sensitive immunoperoxidase techniques. If a difference in expression of CIg is found consistently in larger materials of the two types of BL, it may imply that expression of CIg is influenced by EBV.

The rapid increase in available monoclonal antibodies has made a more detailed subclassification of lymphomas possible. Monoclonal antibodies such as 38.13,[40] or others recognizing more or less BL cell–specific antigens, will undoubtedly lead to an improved understanding of the taxonomic position of BL and may disclose the counterpart to the BL cell in normal lymphoid tissues. Such antibodies may become valuable diagnostic tools for immunopathologists in the differential diagnosis of high-grade malignant lymphomas. Since it is now possible to perform most cell marker studies *in situ* in cryostat sections of fresh-frozen tissue samples using the immunoperoxidase technique, the pathologist should secure and store frozen tissue samples whenever a case of BL is suspected. If stored at −70°C in air-tight packing, the membrane antigens can be studied years later, when new and relevant antibodies certainly will become available.

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CHAPTER 7

Burkitt's Lymphoma: Clinical Aspects and Treatment

Ian Magrath

Epidemiology and Epstein-Barr Virus Association

At the present time the diagnosis of Burkitt's lymphoma (BL) is made on the basis of the histologic and cytologic appearance of the tumor, described definitively by a World Health Organization (WHO) panel in 1969.[13] It seems probable, however, that this morphologic appearance encompasses more than one pathologic entity, for a number of differences exist between BL in Africa and BL in North America that are not explicable on the basis of racial differences alone.[14,77]

BL in the high-incidence area of Africa—roughly 15° north and south of the equator (Figure 7–1)—occurs in 5–15 children/100,000 and accounts for close to one-half of all childhood cancer. The incidence varies quite markedly even within the so-called endemic area of Equatorial Africa, but based on the lower estimate, the disease is about as prevalent as acute lymphoblastic leukemia in children 3–5 years of age in the United States. In Europe and the United States, BL appears to have a similar incidence, and probably accounts for about one-sixth of childhood lymphomas, i.e., 30%–50% of non-Hodgkin's lymphomas. Because of very marked variations in diagnostic practices, precise incidence figures are difficult to obtain. Indeed, in various published series of childhood non-Hodgkin's lymphoma, the frequency of BL varies from 19% to 57%.[35,45,109] Some centers do not make the diagnosis at all. However, based on available information, the average annual incidence of BL in children below 16 years of age in western countries is about 0.1–0.3/100,000. This is 20- to 100-fold lower than the incidence in Equatorial Africa. Moreover, blacks appear to be underrepresented in BL series in the United States; for example, only about 3% of patients treated at the National Cancer Institute (NCI) and 5% of patients in the BL registry[72] are Black.

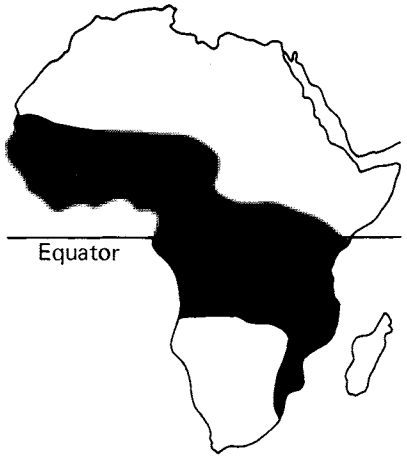


FIGURE 7–1 Map showing the high-incidence area of Burkitt's lymphoma in Africa (shaded area). (Reproduced by kind permission of Livingston Press.)

Whereas a difference in incidence alone does not necessarily indicate a difference in etiology, the marked geographicoclimatic dependence of the distribution of the tumor (Figure 7–1) in Africa[23,27] has not been observed in North America.[72] In Africa the tumor occurs only in areas with a mean monthly temperature above 60°F. In East Africa this is manifested as an altitude barrier (5000 feet at the equator) above which

the disease does not occur. The altitude barrier progressively lessens as the distance from the equator increases. BL is also rare in arid regions and requires a relatively uniform distribution of at least 20 inches of rainfall throughout the year. The limitations imposed by rainfall are most obvious in West Africa. It was the observation of these climatic determinants which led to the suggestion that the disease in Africa may be spread by an insect vector,[51] and, in view of the known viral etiology of a number of animal tumors, that BL may be induced by a horizontally transmitted virus.

The search for a viral etiology led directly to the discovery of Epstein-Barr virus (EBV)[40] and subsequently to the observation that EBV DNA is present in the tumor cells of some 96% of African cases but in only about 10%–20% of American cases.[4,74,77] This difference is emphasized by the fact that only about 16%–25% of American patients with BL lack antibodies to EBV.[72,77] Thus, many seropositive patients have tumors in which the EBV genome is not detected. This also applies to the small number of African EBV genome–negative cases that have been described.[104] EBV genome–negative and –positive Burkitt's tumors must therefore differ at least with regard to the susceptibility of the cells to EBV infection—a contention that is supported by the lack of EBV receptors on cell lines derived from EBV genome–negative cases.[83] It is of interest that the incidence of EBV genome–negative cases in Equatorial Africa appears to be similar to the overall incidence of BL in North America (approximately 0.2/100,000). This is consistent with the possibility that EBV genome–negative [or EBV nuclear antigen (EBNA)-negative] BL occurs with similar incidence in Africa and America, and probably throughout the world, whereas EBV genome–positive BL is a climate-dependent tumor that has a high frequency in Equatorial Africa and is extremely rare outside Africa.

The association of EBV with BL may be even more complex than suggested by the above discussion, because two other groups of patients with EBV DNA–containing tumors have recently been described. The first is North African patients, studied in France,[107,108] and the second is male homosexuals.[146] The finding of EBV DNA–containing BL in North Africa clearly calls for an intensive effort to study the EBV association of tumors from countries outside Africa, the United States, and Europe. Further studies on North African cases to determine the incidence and to establish whether the distribution is climate dependent are also clearly warranted.

At present, the diagnosis of BL has been made in seven male homosexuals in North America. Four of 5 tumors tested contained the EBNA, indicating the presence of EBV DNA.[146] That male homosexuals should develop malignant lymphomas is not surprising in view of the observation of a profound immunodeficiency syndrome in a high proportion of such individuals. In its severest form, this syndrome results in essentially complete lymphopenia with consequent multiple opportunistic infections. Patients with less severe degrees of the syndrome may develop malignant tumors, particularly disseminated Kaposi's sarcoma.[30] These patients are comparable in some ways to renal transplant recipients and patients with certain forms of congenital immunodeficiency. Such immunodeficient individuals have a much greater tendency (up to 300 times) than normal individuals to develop malignant lymphomas.[55,65,77,106] In general, however, the lymphomas of congenitally or iatrogenically immunosuppressed patients have been classified as large cell or immunoblastic lymphomas rather than BL, and recent evidence strongly suggests that the majority of these tumors result as a consequence of the escape of EBV-containing lymphocytes from the normally strict control, as a direct consequence of immunosuppression.[78] This notion is supported by the similarity of some of these syndromes to fatal infectious mononucleosis, as well as by the polyclonal nature of the lymphoproliferative process and the presence of EBV in the proliferating cells.[53]

The relationship between lymphomas occurring in immunosuppressed patients with lymphoproliferative syndromes and the malignant lymphomas of male homosexuals remains to be seen, but it is worthy of note that malignant lymphomas diagnosed as BL have also been described in a sex-linked immunodeficiency syndrome, described by Purtilo et al., associated with an inadequate ability to regulate EBV-infected cells.[112,113] Further studies are needed before it will become clear whether these tumors would be accepted as BL by all pathologists (the diagnosis in some of the 7 tumors mentioned above is disputed), or, even if this proves to be the case, whether they can be shown at a karyotypic and, ultimately, molecular level to be identical to other types of BL.

TABLE 7–1 Anti-EBV Antibodies in Patients with Various Diseases and Control Groups

Disease	Anti–Virus Capsid Antigen			Anti–Early Antigen[a]		
	No.	Geometric Mean Titer	Titer ≥1:160 (%)	No.	Geometric Mean Titer	Titer ≥1:160 (%)
African Burkitt's lymphoma	391	1:376	87	295	1:111	80
Controls	1401	1:34	12	1401	1:16	3
Infectious mononucleosis	251	1:162	68	203	1:33	65[b]
Controls	201	1:42	9	201	1:17	5
Nasopharyngeal carcinoma	418	1:350	86	255	1:110	81
Controls	422	1:37	12	—	—	—
Hodgkin's disease	489	1:105	40	458	1:30	30
Controls	294	1:55	14	—	—	—
Chronic lymphocytic leukemia	235	1:110	45	235	1:46	46
Controls	116	1:51	13	116	1:25	5

Data from Henle and Henle.[54]

[a] Antibodies against the EBV early antigen are predominantly against restricted (to the cytoplasm) early antigen in Burkitt's lymphoma, and diffuse (throughout the cell) early antigen in nasopharyngeal carcinoma, infectious mononucleosis, Hodgkin's disease, and chronic lymphocytic leukemia.

[b] Other studies indicate an anti–diffuse early antigen response in as much as 85% of patients with infectious mononucleosis.

The possibility that BL may arise preferentially in immunosuppressed individuals has been raised before in the context of African BL. The similarity of the distribution of BL to holoendemic malaria led to the suggestion that malaria predisposes to the development of the tumor, possibly by virtue of its effects on the immune system.[51] This could explain the climatic association of BL. However, a causative role for another environmental agent, e.g., a retrovirus, cannot yet be discounted; if this is the case, it may well be revealed in the near future as numerous investigators examine BL cells for increased oncogene expression.

The possibility that EBV is of etiologic importance in EBNA-positive BL has been provided with little additional support in recent years. It has been shown that patients with African BL have eight- to tenfold higher geometric mean titers of anti–virus capsid antigen antibody levels against EBV, and also higher titers of antibodies to the early antigen (Table 7–1), especially the restricted component;[54] however, this could be a consequence of immunosuppression induced by the tumor,[75,79] or of the presence of EBV antigens (regardless of any etiologic role) in the tumor cells. The latter may also explain the correlation of antibody (to early antigen) titers with clinical course in African patients.[54,84] It is possible that individuals destined to develop BL are less able to "contain" EBV infection. Some support for this possibility is provided by the results of a prospective study in Uganda, which demonstrated higher anti-EBV antibody levels in individuals who later developed BL than in controls. Although this was interpreted as indicating an etiologic role for EBV,[46] it is also possible that this reflects relative immunologic "deficiency" in such patients (including a lack of suppressor cells for EBV-infected cells or for EBV-antibody–producing cells) and a resultant predisposition to BL. African children have a seroconversion to EBV seropositivity much earlier than controls in the United States[54] (Figure 7–2);

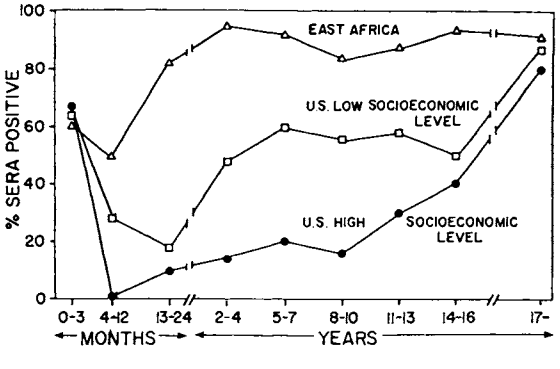


FIGURE 7–2 Anti-virus capsid antigen (of EBV) titers in East African children and American children from different social strata. The differing age dependency of seroconversion in the three groups is well demonstrated. Whether early seroconversion predisposes to EBNA-positive Burkitt's lymphoma or simply reflects the socioeconomic status of the population at risk is not known. (Data from Henle and Henle,[54] with permission.)

however, in the context of the high frequency of EBNA-positive BL in Africa, the significance of this finding remains speculative.

Thus the case for EBV remains unproven, and it is likely that this question will not be resolved until molecular studies unravel the influence of the EBV genome on the behavior of BL cells or demonstrate an alternative molecular mechanism pertinent to pathogenesis. This issue is discussed further below. EBV antibodies are not of diagnostic value since elevated levels may occur in a variety of situations, especially immunosuppression. Furthermore, in the United States some patients with BL remain seronegative.[72]

Immunological Characteristics and the Normal Counterpart Cells of BL

BL is classified as a "B-cell" tumor because it expresses surface immunoglobulin. This is almost always of the IgM class, although the presence of additional heavy chain classes (gamma and alpha) on the surface has been described, and rare tumors express only gamma or alpha.[50] The tumors are monoclonal—as evidenced by expression of a single glucose-6-phosphate dehydrogenase isoenzyme in female heterozygotes[43] and by the expression of either kappa or lambda light chains. The proportion of kappa to lambda tumors is about 3:1, similar to that of kappa-to-lambda expression by lymphocytes in peripheral blood. Some tumors which fail to express surface immunoglobulin, but which express cytoplasmic immunoglobulin only, have been described. These tumors, although morphologically indistinguishable from BL have, by definition, pre-B rather than B-cell characteristics. The cell line "Raji" derived from a West African BL appears to express predominantly cytoplasmic immunoglobulin, but under certain circumstances can switch to pure surface immunoglobulin synthesis.[49] This suggests that BL is the neoplastic counterpart of a very early B-lymphocyte, rather than the equivalent of a transformed lymphocyte or immunoblast. The neoplastic expression of the immunoblast is probably the large cell lymphomas, some of which are designated "immunoblastic lymphoma," and which have been known in the past as reticulum cell sarcoma or, in the Rappaport classification scheme, "diffuse histiocytic lymphoma."[76]

BL—even those rare tumors with pre-B cell characteristics[44,64]—does not express terminal deoxyribonucleotide transferase (tdT). This clearly differentiates this disease from acute lymphoblastic leukemia with pre-B characteristics, which essentially invariably expresses tdT.[76] It is of interest that after birth pre-B cells are concentrated mainly in the bone marrow (where they account for 1–4% of marrow lymphocytes in adults and probably more in children), and that the majority of normal pre-B cells do not express tdT.[105] A small subpopulation (about 1%) of pre-B cells is tdT positive.[57] Since non-T acute lymphoblastic leukemia (which expresses tdT) has been shown to possess rearranged immunoglobulin genes and therefore to be the neoplastic counterpart of cells committed to B-cell differentiation,[68] it seems probable that tdT positive B-cell precursors are the normal counterpart cells of common acute lymphoblastic leukemia, whereas tdT negative pre-B cells and the earliest surface immunoglobulin-bearing cells are the normal counterparts of BL, which usually expresses surface immunoglobulin. A proposed schema of the neoplasms of early B-cells is shown in Figure 7–3.

It is known that the earliest B-cells (i.e., by definition, surface immunoglobulin-bearing cells) express IgM, and that as they differentiate and complement, EBV and Fc receptors can also be detected on the cell surface.[14,77] This step—the development of EBV receptors—may be as crucial to the distinction between African and American BL as is tdT to the distinction between "lymphoblastic" malignancies (including leukemia and lymphoma) and BL. Although no conclusions can be drawn regarding the possible etiological role of EBV in African (or, more specifically, EBNA positive) BL, the almost complete lack of EBV receptors in American BL—deduced by the lack of such receptors in derived cell lines rather than direct measurements—must surely account for the absence of EBNA or evidence of EBV DNA in this tumor.[14,76,83] Cell lines derived from African BL express EBV receptors, as would be anticipated.[83]

One apparently discordant characteristic of American versus African derived cell lines detected recently is that quite large quantities of IgM have been shown to be secreted by American lines, but not African.[11] This is not an *in vitro* artifact, since fresh tumor cells from American patients also secrete IgM and, moreover, monoclonal IgM bands have also been demonstrated in the serum

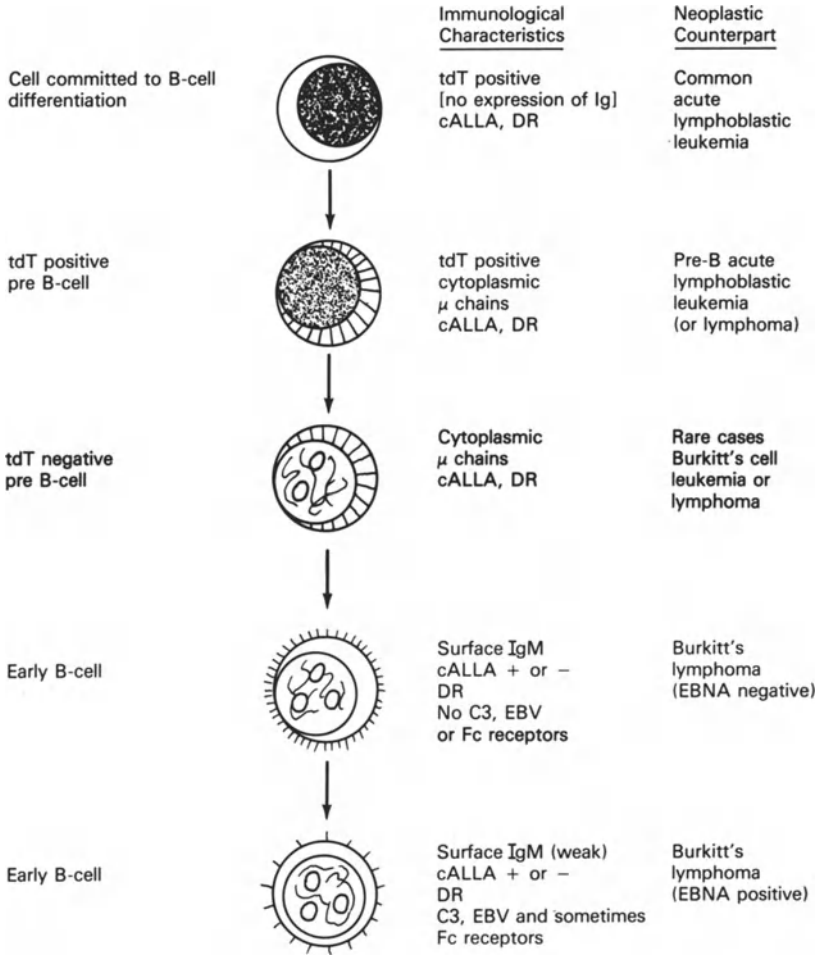

Immunological Characteristics
Neoplastic Counterpart
Cell committed to B-cell differentiation
tdT positive [no expression of Ig] cALLA, DR
Common acute lymphoblastic leukemia
tdT positive pre B-cell
tdT positive cytoplasmic μ chains cALLA, DR
Pre-B acute lymphoblastic leukemia (or lymphoma)
tdT negative pre B-cell
Cytoplasmic μ chains cALLA, DR
Rare cases Burkitt's cell leukemia or lymphoma
Early B-cell
Surface IgM cALLA + or − DR No C3, EBV or Fc receptors
Burkitt's lymphoma (EBNA negative)
Early B-cell
Surface IgM (weak) cALLA + or − DR C3, EBV and sometimes Fc receptors
Burkitt's lymphoma (EBNA positive)

FIGURE 7–3 Probable differentiation sequence of B-cell precursors and early B-cells and their presumptive relationship to acute lymphoblastic leukemia and Burkitt's lymphoma.

of patients with extensive BL or Burkitt's cell leukemia.[85] Although this would at first sight appear to indicate an origin from a more differentiated cell type, this may not be the case, since even pre-B cells have been shown to secrete free heavy chains.[73] Further characterization of the earliest normal B-cells is required before the significance of this finding becomes apparent.

Although surface IgM is by far the predominant surface immunoglobulin isotype, since BL occurs so commonly in the bowel, especially in American patients, it is tempting to speculate that the cells may be neoplastic transformants of cells ultimately destined to become IgA-secreting cells of the gut-associated lymphoid tissue. Only studies of differentiation induction, or possibly of immunoglobulin gene rearrangements, may provide an answer to this question.

Other surface markers of BL cells which do not differ in African and American tumors include the common acute lymphoblastic leukemia antigen, cALLA, which is present in approximately 50% of cases, HLA and DR locus antigens, β-2-microglobulin, and several other B-cell antigens including BAl and Bl.[1,90,94]

Cytogenetics

Whereas a case has been made for separating Burkitt's lymphoma into EBV DNA positive and negative subtypes, certain karyotypic abnormalities are common to both these tumors.[38,134] The most frequent of these is a reciprocal translocation involving the long arm of the eighth chromosome (8q−) (Figure 7–4). Chromosome banding techniques have demonstrated the consistancy of the break point on chromosome 8 (band q24), and it has been suggested that this particular karyotypic abnormality may be essential to the genesis of

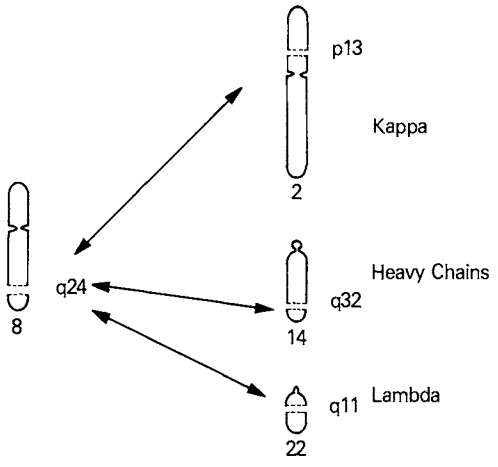


FIGURE 7–4 Schematic diagram showing karyotypic abnormalities in Burkitt's lymphoma. Band regions of break points are indicated. Modified from Rowley.[118]

BL.[67,118] Some cases of BL have been described in the United States, however, in which this abnormality was not present,[38,39] and further, even when present it is not identified in all mitoses.[134] However, only a few mitoses of variable quality are available from most fresh tumors, so the translocation may be overlooked in some cells.

The 8q− is most frequently associated with the addition of chromatin to the long arm of chromosome 14 (14q+), i.e., there is an 8:14 translocation (t8:14). Translocations involving chromosomes 2 and 22 have also been described (in both EBV DNA positive and negative cases) in about 20% of cases studied in France.[16,17,109] The recent demonstration that the heavy chain immunoglobulin genes are located in the q32 region of 14q—precisely the observed break point in BL[66]—while kappa genes are located on chromosome 2 (band p12–13) and lambda genes on chromosome 22 (band q 11)[41,87,89] cannot be mere coincidence in a B-cell tumor. These findings are particularly exciting in view of recent progress in the understanding of oncogenes.[9] It is possible to hypothesize, for example, that an oncogene resides in chromosome 8 which, when activated in a specific type of lymphoid cell, gives rise to BL. The activation could be a result of the chance insertion of viral information, whether of EBV or retrovirus origin, adjacent to the oncogene with consequent promotion of oncogene transcription, or of the transposition of an active promotor region from another chromosome onto chromosome 8, upstream (5|) of the ocogene. It is tempting to speculate that immunoglobulin gene enhancers, which are clearly active in differentiating B cells, may be translocated from chromosomes 14, 2, or 22 to chromosome 8. Support for this possibility has recently been obtained by Erikson and colleagues, who have shown translocation of immunoglobulin variable region genes onto chromosome 8 in Daudi cells.[42] Alternatively, an oncogene from chromosome 8 may be moved to a chromosomal region being actively transcribed, i.e., the region of the immunoglobulin genes on one of the other three chromosomes.[1]

The alternative, but less likely, possibility—that these karyotypic changes are secondary rather than primary—must be considered. It would not be difficult to envisage a heightened susceptibility to chromosomal breakage at specific regions in specific cell types (depending upon recombinational events which are in progress by virtue of normal differentiation). Again, the frequent involvement of immunoglobulin genes in B cell tumors would be quite consistent with this possibility, particularly since it is known that genetic rearrangement of immunoglobulin genes occurs during differentiation.[37] Studies of these possibilities and being pursued in a number of laboratories, aided considerably by the large number of cell lines available from BL.

Cytokinetic Considerations

BL is one of the most rapidly proliferating human neoplasms. This is borne out both by the generally short duration of symptoms (weeks or, at most, months) prior to presentation, the rapid progression which can occur even in the course of a few days while under medical observation, and both *in vivo* and *in vitro* measurements of cellular proliferation. In *in vivo* studies of the growth of subcutaneous masses, a mean actual doubling time of 66 hours (range 38–117 hr) was measured.[56] In *in vitro* studies of thymidine uptake a potential doubling time of 26 hr (range 12–70 hr) was observed.[56] The difference between potential and actual doubling time was accounted for by a high spontaneous cell-loss rate of about 70%. This is also quite consistent with the high propensity to pretreatment uric acid nephropathy from cell lysis in patients with extensive BL.[130]

DNA content histograms indicate that up to

[1] *N.B.:* Since this chapter was completed, it has been shown that the c-myc oncogene resides on chromosome 8(q24 → ter) and is actually translocated in at least some cases into the immunoglobulin heavy chain constant region. The expression of c-myc has also been shown to be slightly increased, i.e., 2–5-fold, compared to normal lymphoblastoid cell lines.

27% of cells may be in S-phase, while labeling indices of between 20% and 60% have been reported.[19,93] Thus, the growth fraction in BL is extremely high (essentially 100%), a factor which may account both for the dramatic initial responses to chemotherapy which are observed and also the long-term remissions—almost certainly cures—which are now seen in a high proportion of patients with BL, including some with very extensive disease.

Clinical Characteristics

Age and Sex Distribution

BL occurs predominantly in young people. In Africa the peak incidence is between 5 and 10 years of age,[26] and in the United States it is quite similar, although there is a rather broader peak between 4 and 12 years of age according to the NCI BL registry.[72] The tumor is more common in males, the overall male to female ratio being 2:1 in Africa,[26] 2–3:1 in the United States,[72,77] and 3.7:1 in France.[109] Certain sites of involvement occur more frequently in one or the other sex, or at certain ages, but these findings are not consistent between Africa and the United States. For example, jaw tumors are relatively more frequent in young males in Africa,[24] whereas in the United States they are relatively more common in older females.[121] Involvement of the bowel in the right iliac fossa (i.e., in the terminal ileum or cecum) is a common presentation of children and adolescents in the United States, but occurs six times more frequently in males.[58] Breast involvement occurs predominantly during or after the second decade.[125]

Anatomic Distribution

BL predominantly involves extranodal sites in both African and non-African cases, although significant differences may be observed in the relative frequency of involvement of various sites between Africa and other countries (Table 7–2).[77] In Af-

TABLE 7–2 Comparison of Disease Sites at Presentation in African and American Burkitt's Lymphoma Patients

	African		American	
Site[a]	No.	%	No.	%
Abdomen (all)	130	58	78	91
Ascites		—	19	(39)[b]
Hepatosplenomegaly	25	11	15	(31)
Right iliac fossa mass		—	8	(17)
Bowel		—	26	(54)
Stomach		—	4	(8)
Ovary		—	7	(15)
Kidney		—	7	(15)
Retroperitoneum		—	8	(17)
Pelvis		—	13	(15)
Unspecified		—	30	(38)
Pleural effusion	7	3	16	19
Bone marrow	16	7	17	20
Peripheral nodes	9	4	11	13
Bone	18	8	8	9
Cerebrospinal fluid/Central Nervous System	43	19	12	14
Paraspinal	38	17	2	2
Testis	4	2	5	6
Pharynx	0	0	9	10
Jaw	136	58	6	7
Mediastinum	1	0.5	3	3
Orbit	25	11	1	1
Other[c]	38	17	7	15
Total patients	224		86	

[a] Many patients had multiple sites of disease.
[b] Percentage of those with abdominal involvement is indicated in parentheses.
[c] Includes thyroid, breast, skin, shoulder, and thigh.

rica, jaw involvement is very common and gives rise to characteristic clinical findings that led to the original recognition of the syndrome throughout Equatorial Africa in the late 1950s.[22] Jaw tumors are more common in males and in young children in Africa; for example, almost all children of 3 years with BL have jaw tumors. The precise site of origin of jaw tumors is not known, but typically there is involvement of the developing premolar and molar teeth, resulting in the characteristic radiologic appearance with early loss of lamina dura, widening of the dental papillae, and lytic changes in surrounding bone.[2,3] Clinically, the teeth tend to be loose and may be displaced or even lost, sometimes followed by premature eruption of permanent teeth. The expansile lesion causes gingival and sometimes gross facial swelling, but ulceration through the skin is extremely rare unless surgical intervention is attempted. In Africa the maxillae are involved about twice as often as the mandibles when clinical examination is the sole criterion, although radiologically, frequency of involvement is similar. Multiple jaw quadrant involvement is seen by x-ray examination in up to 80% of patients with jaw involvement.[2,26] These features differ quite considerably from those of the relatively small number (7%–18%) of American patients with jaw tumors.[121] Adult American patients usually present with toothache, often leading to a diagnosis of abscess and consequent antibiotic treatment. Children may have loose teeth, but the jaw tumors are rarely as extensive as in Africa, mandibular involvement is more frequent than maxillary, and multiple jaw quadrant involvement is uncommon (Table 7–3). In addition, there is no age dependency of jaw tumors, nor is there greater frequency in males in American patients. In fact, jaw tumors occur relatively more frequently in adult females.

Orbital involvement is also relatively common (18% of facial tumors) in African patients; it has been suggested that in these patients it arises by direct invasion from maxillary tumor. This is not always the case, however, for orbital involvement may be unassociated with clinical jaw involvement or any bony changes on x-ray. The tumor remains extraocular until it is very advanced.[26] Outside Africa, orbital involvement is extremely rare. We have seen only one case in 86 patients in the NCI Pediatric Oncology Branch series. Involvement of the salivary glands and thyroid is also seen occasionally in African patients[24] but very rarely in the United States.

In both Africa and the United States, intra-abdominal disease is very frequent.[24,77] In Africa this more often involves the retroperitoneum, mesentary, and omentum than the bowel itself, so that presentation as intussusception, perforation, or intestinal obstruction is rare, whereas in the United States about 20% of patients have such presentations.[58]

The kidneys, and in females, the ovaries, are quite frequent sites of involvement; the adrenals, pancreas, and liver are involved less often, and the spleen, rather uncommonly. Ascites is often present due to peritoneal infiltration. Obstruction of the inferior vena cava or iliac vessels can occur, and this may produce marked dependent edema of the lower limbs because of additional obstruction to collateral vessels. Involvement of the pharynx has not been described in Africans but occurs occasionally in American patients. Sometimes it presents merely as tonsillar enlargement, whereas on other occasions it is severe enough to threaten acute airway obstruction.

Central nervous system (CNS) involvement, manifested as meningeal infiltration with malignant pleocytosis in the cerebrospinal fluid, cranial nerve palsies, or epidural mass with cord compression (usually unassociated with bony changes), occurs in about 30% of African patients at presentation and about 20% of American patients (Table 7–2).[122] Paraspinal soft tissue masses may be detected radiologically.[33] Epidural tumor leading to cord compression, a presenting feature of 15% of African patients, is rare in American patients. It is of interest that at presentation malignant cerebrospinal fluid pleocytosis is documented in only some 25% of African patients who present with cranial nerve palsies and possibly a similar fraction of American patients, although numbers are too small to be sure. At relapse the two findings are associated about 75% of the time. Diffuse infiltra-

TABLE 7–3 Clinical and Radiologic Findings in 18 American Patients with Jaw Tumors

Tumor	No. of Patients
Single mandibular tumor	7
Single maxillary tumors	5
Bilateral mandibular tumors	4
Four jaw quadrant tumors	2

tion of the brain or the development of brain masses is uncommon but has been documented at relapse, usually after repeated CNS recurrences.[82]

In American patients, CNS disease at presentation is associated with bone marrow involvement in 67% of cases.[122] Most patients with CNS involvement have extensive disease elsewhere, although occasionally this is not the case. One of our patients had cervical lymphadenopathy and CNS disease, and another had only a pharyngeal mass. We have not seen CNS disease at any time in 15 patients with completely resected tumors, although nearly all had CNS prophylaxis.[122] In Africa, CNS disease is often associated with extra-abdominal (usually jaw) tumors, and rarely with bone marrow infiltration.

A variety of other sites may be involved, including the pleurae—with resultant effusions (evidence of intrapulmonary involvement prior to death is extremely rare)—testes, and bone marrow. The greater frequency of bone marrow involvement in American patients compared to African patients is reflected both at presentation, where marrow involvement is about three times as common, and at relapse.[86] Whereas in Africa marrow involvement occurs in less than 10% of patients even after multiple relapses, about 66% of American patients who relapse ultimately develop bone marrow involvement. Peripheral lymph node involvement is not common but can be the site of presentation in up to 20% of patients outside Africa. This mode of presentation occurs in less than 1% of African patients.[26] Bone (single or multiple), pericardial (with effusion), or even myocardial and breast involvement occur occasionally in both Africa and elsewhere.

Burkitt's Cell Leukemia (L_3 Acute Leukemia)

The presentation of BL outside Africa with generalized lymphadenopathy, diffuse bone marrow infiltration, and hepatosplenomegaly, often with circulating tumor cells but without mass lesions in other organs, has also been classified as acute lymphoblastic leukemia of the L_3 subgroup (using the French–American–British designation). The L_3 subgroup is characterized by blast cell morphology indistinguishable from BL.[12] This disease, because of its rarity, has not been well characterized, but the surface characteristics (the most important being immunoglobulin which is essentially always IgM) and karyotypic findings (t8:14 or 14q+ abnormalities have usually been detected when sought) are also indistinguishable from those of BL.[15,44,64,115] Rare cases that lack surface immunoglobulin but express cytoplasmic immunoglobulin have been described.[44] It would seem appropriate at present to consider this presentation as simply a varient of BL rather than acute lymphoblastic leukemia.[12] The biologic characteristics of the cells that result in specific patterns of anatomic distribution have yet to be defined, but there is no reason to assume that the designations "leukemia" and "lymphoma" signify generic differences, although the anatomic location may indicate that there are qualitative and/or quantitative differences in cell biology between this and BL presenting in the abdomen. Diffuse marrow involvement presumably also signifies a high tumor burden.

The very poor prognosis usually seen in patients with L_3 or BL cell leukemia may not differ greatly from the prognosis of other patients with very extensive tumor, although considerably more data are needed to establish this. Therefore, whether or not the biologic differences in the tumor cells result in a different response rate to chemotherapy remains to be seen.

Staging

The value of staging as a means of separating different prognostic groups is clear. Unfortunately, comparison of treatment results obtained in different centers is marred by the use of different staging schemes. In malignant lymphomas the prototype staging systems are those devised for Hodgkin's disease; the importance of radiotherapy in its management has rendered detection of all sites of disease of critical importance. The value of staging as a prognostic indicator in Hodgkin's disease is much less apparent now than 10 years ago, because advances in treatment have so markedly improved results that even patients with advanced disease can enjoy prolonged survival.[116] Nonetheless, as long as radiotherapy continues to be used as the sole treatment modality in Hodgkin's disease in at least some patients, or different stages are treated differently, detailed staging will continue to be essential to good management.

There are a number of differences between Hodgkin's disease and BL, however, which have important implications for the most appropriate staging scheme. The first is that radiotherapy has

a small role at best in the management of BL, therefore, precise staging is less important in its management. The second is that BL is predominantly extranodal, whereas Hodgkin's disease is a disease of lymph nodes. Thus, whereas well-characterized patterns of spread in Hodgkin's disease assist with regard to the planning of both a rational investigation of disease extent and radiotherapeutic strategems, in BL neither applies. It would seem then, that Hodgkin's disease staging systems do not represent a good model for BL.

There is good evidence that prognosis in BL is largely dependent upon the total tumor burden.[84] Thus, staging systems should emphasize tumor burden rather than disease sites (although the two cannot be completely separated). In Africa, a very clear distinction can be drawn between patients who present with jaw tumors alone (small tumor burden) and those with intra-abdominal disease (large tumor burden) whether or not this is associated with jaw tumors.[81,103] This is reflected in most of the staging schemes that have been proposed for African BL (Table 7–4). Further refinement of these two major divisions led to the staging system proposed originally by Magrath et al. (Table 7–4),[81,138] which also took account of the excellent prognosis enjoyed by patients in whom all abdominal disease was surgically resected prior to chemotherapy.

Although CNS and bone marrow involvement have been considered in both Africa and the United States to signify a poor prognosis, with

TABLE 7–4 Staging Schemes for African Burkitt's Lymphoma

Author[a]	Stage	Definition
Morrow et al.	I	One anatomic region involved; two contiguous regions on same side of diaphragm
	II	Two or more noncontiguous regions; more than two contiguous regions (in both cases, on one side of the diaphragm)
	III	Intrathoracic, intra-abdominal, intracranial, or bone marrow involvement, or anything more than I or II
Clifford et al.	I	One anatomic area involved
	II	Two contiguous areas; two or more areas on the same side of the diaphragm
	III	Both sides of the diaphragm or bone marrow involvement
	IV	CNS involvement
Ziegler et al.	I	Single facial tumor
	II	Two or more separate facial tumors
	III	Intrathoracic, intra-abdominal, paraspinal, or osseous tumor (except facial)
	IV	CNS (malignant cells in the cerebrospinal fluid) or bone marrow involvement
Ngu	I	One jaw quadrant involved
	II	Two or more jaw quadrants involved
	III	Intra-abdominal or osseous tumor
	IV	CNS (brain, cord, or meninges) involvement
Williams	I	Tumors above clavicular line without cranial or spinal neuropathies
	II	Tumors below clavicular line without cranial or spinal neuropathies
	III	Cranial or spinal neuropathies or osseous tumors
	IV	CNS or bone marrow involvement or death within 2 weeks
Magrath et al.[8 b]	I	Single extra-abdominal tumor
	II	Multiple extra-abdominal tumors
	III	Intra-abdominal tumor with or without a single jaw tumor
	IV	Intra-abdominal tumor with extra-abdominal sites other than a single jaw tumor
	IR	Completely resected intra-abdominal tumor without extra-abdominal tumor

[a] For source material see Ziegler and Magrath,[138] from which this table is adapted.
[b] Designations subsequently modified to A, B, C, D, AR (see Ziegler and Magrath[138]). ABCD system is used in this chapter.

TABLE 7–5 Staging Schemes for non-African Burkitt's Lymphoma

Author	Stage	Definition
Wollner et al.[132]	I	One site
	II	Two or more sites either above or below the diaphragm
	III	Two or more sites above and below diaphragm; extensive intrathoracic involvement; extensive intra-abdominal involvement
	IV	CNS or bone marrow involvement
Murphy et al.[92]	I	Single tumor (extranodal) or single anatomic area (nodal) apart from mediastinum or abdomen (extranodal)
	II	Single tumor (extranodal) with regional node involvement; two or more nodal areas on the same side of the diaphragm; two single (extranodal) tumors with or without regional node involvement on the same side of the diaphragm; primary gastrointestinal tumor (usually ileocecal) with or without involvement of associated mesenteric nodes only
	III	Two single tumors (extranodal) on opposite sides of the diaphragm; two or more nodal areas above and below the diaphragm; all primary intrathoracic tumors (mediastinal, pleural, thymic); all extensive primary intra-abdominal disease; all paraspinal or epidural tumors regardless of other sites
	IV	Any of the above with initial CNS or bone marrow involvement

Wollner et al.[132]; Murphy.[91]

intrathecal therapy many African patients with CNS disease can achieve long-term survival.[145] Patients with extraabdominal disease and CNS involvement, for example, have a good prognosis, although patients with extensive intra-abdominal disease and CNS involvement have a very poor prognosis.[84,103,138] Furthermore, since diffuse bone marrow involvement is very uncommon in African patients and its importance as a prognostic determinant in any case appears to be similar to that of CNS disease, the presence of either bone marrow involvement or CNS disease per se does not appear to characterize the worst prognostic group (highest stage).[80] In the United States, the importance of bone marrow involvement as a prognostic determinant is also somewhat confused by the arbitrary distinctions between leukemia and lymphoma which have been applied; these are based upon the extent of bone marrow involvement. In many centers, patients with greater than 25% blast cells in the bone marrow are diagnosed as having leukemia and are excluded from descriptive series or therapeutic studies[92,132] Nonetheless, patients with bone marrow involvement do appear to have a poor prognosis.

The tendency in the United States to lump all childhood malignant lymphomas together as "non-Hodgkin's lymphoma" (particularly before the descriptions of lymphoblastic lymphomas of T cell origin) has also very much clouded the issue of the importance of bone marrow or CNS disease in BL.[92] The two main staging schemes for childhood non-Hodgkin's lymphoma (Table 7–5) are in fact based upon prognostic factors for both T cell (lymphoblastic) and B cell (undifferentiated and diffuse histiocytic) lymphomas, which are very different tumors in terms of their clinical behavior.[77,92,132] It is therefore perhaps surprising that these staging schemes—modified from Hodgkin's disease and applied to both B and T cell lymphomas—have shown as much clinical relevance as they have in BL. It now behooves us to develop rational staging schemes for individual pathologic entities, based upon prognostic factors for that specific entity. Even the scheme derived

TABLE 7–6 Proposed Staging Scheme for American Burkitt's Lymphoma

Stage	Definition
I	Single extra–extra-abdominal site
IR	Resected (>90%) intra-abdominal tumor
II	Multiple extra-abdominal sites excluding bone marrow and CNS
III	Unresected intra-abdominal disease; intra- and extra-abdominal disease (except bone marrow); extra-abdominal sites including bone marrow and/or CNS
IV	Intra-abdominal and bone marrow involvement regardless of other sites

for African BL may not be appropriate for BL in other parts of the world. A proposed staging system for EBNA-negative BL, based on the results obtained at the NCI Pediatric Oncology Branch, is shown in Table 7–6.

Investigations

In investigating the patient with suspected or verified BL, several considerations should be borne in mind. First, it is essential to determine the presence of potentially life-threatening complications resulting from compressive or obstructive lesions, or the presence of tumor involvement of sites that could lead to permanent physical disability if not dealt with as a medical emergency (Table 7–7). The major complications of this kind are airway obstruction from pharyngeal tumor, massive bleeding from ulcerated lesions of the gastrointestinal tract, or the presence of CNS disease involving cranial nerves, meninges, brain parenchyma, or epidural tumor causing spinal cord compression. In addition, the state of hydration, renal function, and the presence of renal tract obstruction must be immediately assessed because of the high incidence of uric acid nephropathy in patients with extensive disease. At the earliest possible time, it is important to initiate definitive diagnostic studies (i.e., histologic and, where possible, immunologic examination of tumor tissue) if these have not already been done; in patients with extensive tumor, delays caused by the processing of biopsy material must be kept to an absolute minimum. Special studies on tumor cells may be required to establish the diagnosis with certainty. The most important of these are the demonstration of surface immunoglobulin, the lack of terminal deoxynucleotidyl transferase (a hallmark of T cell lymphoblastic lymphoma), and the presence of specific cytogenetic abnormalities (see above).[38,77,88,134]

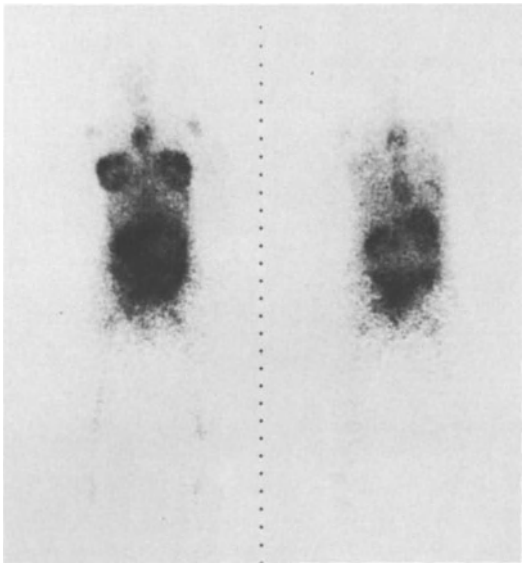
Investigations to determine the extent of tumor include, as always, a good history and physical examination. In most cases, the physical examination alone will reveal whether the tumor burden is large or small; and physical examination—supplemented by examination of the cerebrospinal fluid and bone marrow, assessment of urine output, biochemical parameters of renal and liver function, and a blood count—is sufficient to permit good medical management although this does not represent optimal care. Frequently, in developing countries, sophisticated imaging techniques are not available, and the above studies may then comprise the complete extent of the investigation. Even in such countries, however, a chest x-ray and intravenous pyelogram, to exclude mechanical obstruction to urinary outflow, would normally be performed; where available, more sophisticated imaging techniques can add considerably to the delineation of tumor sites.

Of the various imaging techniques available, perhaps the most useful in BL is the gallium scan (Figure 7–5). Gallium is taken up rapidly and avidly by BL. In a recent review of over 40 cases of B cell lymphomas in young people seen at the NCI[86], in which multiple imaging studies were

TABLE 7–7 Some Complications of Lymphomatous Infiltration or Compression Requiring Immediate Intervention

Tumor Location	Potential Complication	Management
Pharynx	Airway obstruction	Tracheostomy, ST[a]
Esophagus or stomach	Dysphagia, bleeding	?Surgery, blood transfusion, ST
Small bowel	Intestinal obstruction; intussusception; bleeding	Surgery (antibiotics), blood transfusion, ST
Large bowel	Intestinal obstruction; bleeding	Surgery (antibiotics), blood transfusion, ST
Urogenital tract	Ureteric obstruction; renal failure	Hydration, ?dialysis, ST
Epidural	Paraplegia	Radiotherapy, ?laminectomy, ST
Pericardial or myocardial	Tamponade	Pericardiocentesis, ST
Bone marrow	Pancytopenia	Blood component transfusions, antibiotics, ST
Retroperitoneum	IVC[b] occlusion; pulmonary embolus	IVC balloon/umbrella, ST

[a] ST: specific therapy.
[b] IVC: inferior vena cava.



a b

FIGURE 7–5 Gallium scan in an 18-year-old female patient showing involvement of breasts, mediastinal nodes, abdomen, and pelvis. (a) Anterior view; (b) posterior view.

performed at the time of presentation, it was observed that abnormalities were not detected in the abdomen by other imaging procedures in the absence of abnormalities on the gallium scan. Thus, computerized axial tomography (CAT scan) and ultrasound examinations may be avoided in this circumstance and used only to obtain further specific information concerning the extent or location of intra-abdominal or, rarely, intrathoracic or intracranial masses.[69,124] Ultrasound is particularly useful for determining the presence of hydronephrosis secondary to ureteric obstruction; it is also more useful than the standard CAT scan or liver–spleen scan for detecting intrahepatic masses, although a CAT scan in conjunction with modern liver–spleen contrast materials may be as good. Ultrasound may provide better imaging than a CAT scan in small children, who usually lack sufficient retroperitoneal fat for adequate radiographic delineation of intra-abdominal viscera.[69,124] BL tumor masses frequently appear as cystic structures on gray-scale ultrasound examinations because of their marked echolucency and homogeneity.[124]

The precise anatomic localization possible with CAT scans, particularly in older individuals, makes this study especially useful when a determination must be made as to whether the likelihood that intra-abdominal disease can be completely resected warrants laparotomy. The CAT scan is also the most precise means of delineating intrathoracic disease, and both CAT scan and ultrasound may detect pleural effusions or ascites when present in only very small quantities.

In general, lymphangiography has not been widely used in childhood lymphomas because these tumors are predominantly extranodal. Furthermore, any advantage of lymphangiography over CAT scan, for example, in detecting minimal lymph node disease, is obviated by the relatively trivial clinical significance of such a finding compared, for example, to its importance in Hodgkin's disease.

Other studies may be done in specific circumstances. Barium studies and endoscopy provide the best means of precisely delineating bowel involvement and ulceration but are not performed routinely. Liver–spleen scan has nothing to add to the above investigations, and bone scan will only increase the definition of bone lesions that are also detected by gallium. In the presence of CNS symptomatology and physical signs, a head CAT scan or myelogram may be indicated.

Tumor Markers

Perhaps the most useful and readily available biochemical indicator of tumor burden in BL is the total lactate dehydrogenase (LDH) level.[130] This correlates significantly with clinical stage at presentation (Figure 7–6), and a progressive rise in a patient with previously normal levels may antedate clinical evidence of relapse. Since LDH is nonspecific, its significance is greatest when unaccompanied by elevations in transaminases and when there is a clear, progressive trend. The proportions of LDH isoenzyme fractions in BL cells approximate those of B lymphocytes,[18] and the overall pattern can clearly be distinguished from T lymphocytes (fraction 5 is proportionately higher than 1 in B cells, vice versa in T cells); however, isoenzyme fractionation of serum LDH is unlikely to be of diagnostic significance because the tumor pattern is superimposed and partially obscured by the underlying normal serum pattern. Occasionally, however, isoenzyme fractionation may be of value to distinguish tumor LDH from liver or red cell LDH. The possible value of the absolute level of fraction 4—which appears to be the fraction that changes by the greatest propor-

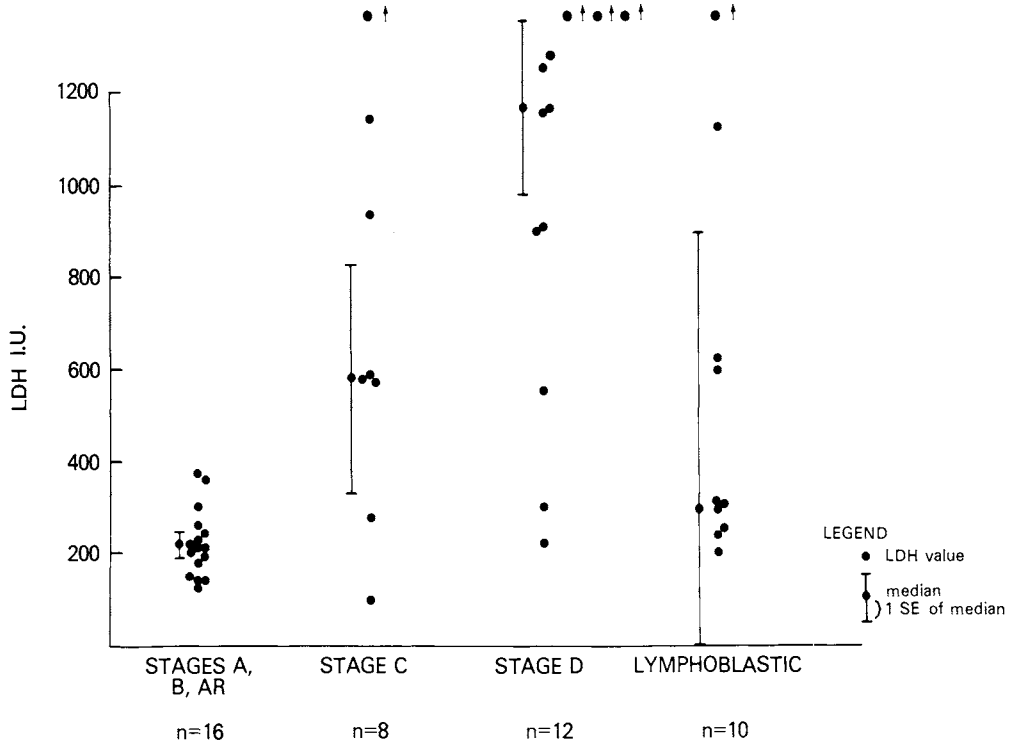


FIGURE 7–6 Correlation of pretreatment LDH levels with stage in patients with undifferentiated lymphomas, predominantly of Burkitt's type. LDH levels in a small group of patients with lymphoblastic lymphoma are also shown.

tion during disease progression or regression—is, however, worthy of further study.[36] A potentially valuable marker, detected by dilute agarose gel electrophoresis of serum, is tumor-secreted immunoglobulin. Monoclonal immunoglobulin bands have been detected in the serum of about 60% of patients with extensive BL or Burkitt's leukemia.[85] Such bands correlate with disease status, and prospective studies are currently underway to determine their utility.

Other tumor markers include urinary β-aminoisobutyric acid or its ratio to β-alanine, and polyamines.[131] β-Aminoisobutyric acid excretion is markedly elevated in patients with a large tumor burden, and usually increases even more during acute tumor lysis (see below), before normalizing during remission. β-Aminoisobutyric acid is derived ultimately from pyrimidine bases, predominantly thymine, but also uridine, and its excretion is presumably, like uric acid, an indicator of nucleic acid breakdown. Polyamines are derived ultimately from ornithine, and are usually elevated in the presence of a large tumor burden. A variety of nucleosides and have been examined as potential tumor markers, but, like β-aminoisobutyric acid and polyamines, require sophisticated biochemical techniques for their measurement and so have not been widely used.

Management

Because of the wide variety of anatomic locations that may be involved by Burkitt's tumor, a correspondingly wide variety of acute clinical situations may develop that require immediate attention prior to the initiation of specific treatment (Table 7–7), or which may occur shortly after the initiation of cytotoxic chemotherapy. In general, although immediate supportive measures may be required, only effective treatment of the underlying tumor will provide more than temporary benefit, and in this extremely rapidly growing tumor, specific therapy must be instituted at the earliest possible opportunity. Even where all overt tumor has been surgically resected, e.g., in abdominal, particularly bowel presentations, inordinate delay in commencing chemotherapy (i.e., more than is required for recovery from the immediate postoperative period—usually 3–5 days) may result in con-

siderable regrowth of tumor and hence loss of any advantage gained by the surgical reduction in tumor volume. In some cases, the commencement of chemotherapy itself constitutes emergency treatment, for example, where tumor masses are resulting in compression of vital or important structures, e.g., upper airway, spinal cord, or ureters.

Biochemical Consequences of Rapid Tumor Growth and Lysis

An additional consequence of the extremely rapid growth rate of Burkitt's tumor—and perhaps also in part due to biochemical idiosyncrasies—is the very high rate of renal and metabolic complications.[7,21,34,130] Azotemia occurs in about one-half of the patients with a large tumor burden (stages C and D in the NCI system, which is identical to that of Magruth et al.[8] (see also Table 7–4), but uses letters instead of numerals) either prior to, or in the week after chemotherapy. These patients almost invariably have a high LDH level (over 600 IU/l). Prior to the initiation of therapy, uric acid nephropathy is the major cause of acute oliguric renal failure—some 50% of uricosemic patients are also azotemic (Table 7–8). This may be compounded by ureteric obstruction or dehydration. Rarely, hypovolemia may be a consequence of marked redistribution of body fluids resulting from massive effusions or inferior vena caval and caval tributory occlusion. The profound lower limb edema which has been observed in this situation may be only compounded by the administration of large quantities of fluid, particularly if unaccompanied by plasma expanders and diuretics. Nevertheless, in the majority of patients, vigorous hydration accompanied by alkalinization is usually sufficient to reduce the uric acid burden considerably. When this treatment is coupled with allopurinol administration (10 mg/kg) to prevent uric acid formation, uric acid levels frequently reach the normal range within 24–48 hours. If necessary, furosemide, or a combination of furosemide and thiazide diuretic may be used to establish a sufficiently brisk diuresis.

The delay in the institution of specific therapy occasioned by the necessity to normalize the serum uric acid level is often acceptable in this circumstance, since this may avert the need for hemodialysis. This may not be the case where mechanical obstruction to urine outflow, or other constraints on renal function exist (e.g., a prerenal component, use of radiopaque dye in the presence of compromised renal function, or massive renal infiltration by tumor), and in such cases hemodialysis may be necessary prior to the initiation of chemotherapy. We have avoided nephrostomy or ureteric catheterization in the presence of ureteric obstruction because of the potentially high risk of compli-

TABLE 7–8 Frequency of Renal and Metabolic Complications in Patients with Burkitt's Lymphoma Treated at the National Cancer Institute

Complication	No./Total[a]	Percentage
Pretreatment uricosemia (Cr. ≥8 mg/dl)	23/67	34
Stages A, B, AR	2/16	12
Stages C, D	21/51	41
Pretreatment azotemia (Cr.[b] ≥1.6 mg/dl)	12/67	18
Stages A, B, AR	0/16	0
Stages C, D	12/51	23
Posttreatment azotemia[c] (Cr. ≥1.6 mg/dl)	9/67	13
Stages A, B, AR	0/16	0
Stages C, D	9/51	18
Posttreatment hyperphosphatemia (≥5.0 mg/dl)	35/67	52
Increase in urinary phosphorus excretion (>twofold)	11/18	61
Ureteric obstruction (all stage C or D)[d]	10/28	36
Azotemia	3/6	50
Nonazotemia	7/22	32
Required hemodialysis	7/67	10

[a] Total includes some patients with recurrent disease.
[b] Cr.: Creatinine.
[c] Only patients not azotemic prior to therapy included.
[d] Intravenous pyelogram findings only included.

cations (infection, or ureteric perforation at a site of tumor involvement).

When chemotherapy is initiated, the extremely rapid tumor lysis that ensues results in further biochemical abnormalities in the majority of patients with a large tumor burden (Figure 7–7). About 13% initially develop azotemia after chemotherapy (Table 7–8), resulting from the increased protein, purine, and phosphate load on the kidneys. The key to the prevention of renal shutdown is the continuation of *vigorous diuresis*—at least 3000 ml fluid/M^2 should be given, and more if tolerated. To maintain an adequate urine output, it may be necessary to use diuretics in some patients (as above). Once the serum uric acid level has been normalized, prior to commencing chemotherapy, it is usually unnecessary to continue vigorous alkalinization; the increase in solubility of xanthine and hypoxanthine achieved by increasing the pH[52] probably does not compensate for the decrease in solubility of phosphates, or for the added risk of tetany and cardiac arrhythmias in the presence of hypocalcemia, which accompanies the hyperphosphatemia.

The precipitation of either purines or phosphates, or both, in the renal tubules may give rise to acute oliguric renal failure following chemotherapy. In the presence of allopurinol, uric acid concentration rarely exceeds the solubility of uric acid in urine, but the xanthine concentration frequently does, and it is therefore advisable that at least some of the purine load is excreted as uric acid, which is considerably more soluble than xanthine at pH 7 (Table 7–9). Phosphate excretion, on the other hand, which is not a problem prior to chemotherapy, may become of great importance subsequently, due to the massive lysis of tumor cells.[130] More than one-half of all patients, includ-

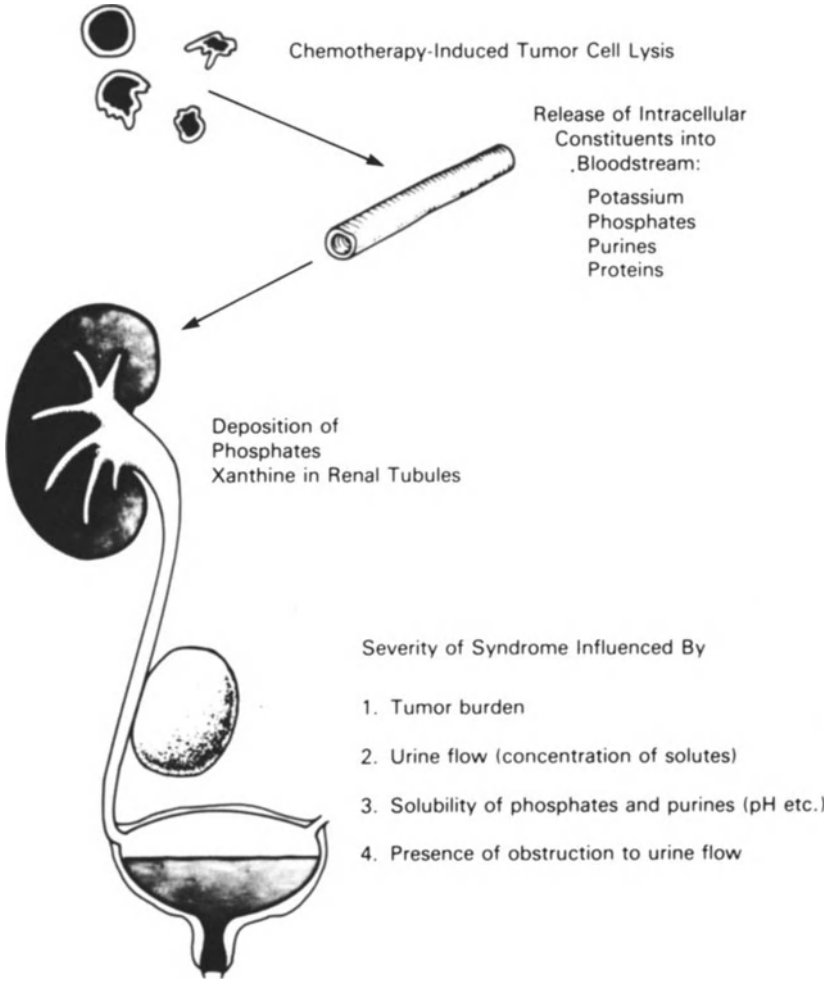


FIGURE 7–7 Pathogenesis of the acute tumor lysis syndrome.

TABLE 7–9 Solubility of Purines in Urine

pH	Xanthine (mg/l)	Hypoxanthine (mg/l)	Uric Acid (mg/l)
5	50	1400	150
7	130	1500	2000

Adapted from Hande et al.[52]

ing many who never develop azotemia, have at least a doubling of urinary phosphorous excretion.[130]

Apart from acute oliguric renal failure, hyperkalemia (with its attendant risk of cardiac arrest) or hypocalcemia (a result of profound hyperphosphatemia) may occur. Hyperkalemia, the fatal consequences of which originally drew attention to the acute tumor lysis syndrome,[7] can develop within hours of the initiation of treatment but does not occur in the presence of an adequate urine flow. Because of this, potassium-containing intravenous solutions are normally avoided during the first 1–2 days of chemotherapy. Hypocalcemia is rarely of sufficient magnitude to give rise to cardiac arrythmias or tetany, but if it does, it should be treated with intravenous calcium only with extreme caution because of the risk of visceral calcium phosphate precipitation. If the calcium phosphate solubility product is likely to be exceeded by intravenous calcium administration, hemodialysis is a safer form of therapy. Hemodialysis is also indicated when serum phosphorus or creatinine levels are rising rapidly, particularly in the presence of a falling urine output, which reduces elimination and increases solute concentration such that a vicious cycle sets in, resulting in very rapid deterioration. If conservative measures have failed or are inappropriate to deal with hyperkalemia or hypocalcemia, hemodialysis may also be indicated.

We have not seen permanent renal impairment as a consequence of the tumor lysis syndrome. Nearly all patients have essentially normal renal function by day 10 of treatment. LDH levels increase shortly after chemotherapy in about one-third of all patients, or one-half of azotemic patients[130]—also as a result of tumor cell lysis. The time course of biochemical changes in 33 patients with lymphomas is shown in Figure 7–8. Whether or not the extent of the biochemical changes occurring after treatment could be used as a measure of tumor burden, or response to treatment, or both, has not been studied.

Specific Therapy

Because of its extremely rapid growth rate, specific therapy for BL should be initiated at the earliest possible time. Delay, for whatever reason, will permit tumor growth and may result in deterioration of the patient's general condition, worsening of complications, and, by virtue of increased tumor bulk, a decrease in the overall chance of survival. Thus, with the exception of the emergency management of complications or the control of uricosemia, there are no valid reasons to delay the initiation of therapy once staging studies have been completed.

Surgery

One important decision that must be made at the completion of staging studies is whether there is a reasonable possibility that the tumor burden can be reduced at least tenfold by surgical resection prior to starting chemotherapy. This decision is largely dependent upon tumor sites. In general, patients with bone marrow or CNS involvement, multiple sites of disease outside the abdomen, or extensive retroperitoneal disease are not considered candidates for surgery. The most suitable patients are those with tumor confined to the bowel, mesentary, ovaries, or one kidney. Residual tumor plaques on the peritoneum, or enlarged mesenteric lymph nodes do not annul the benefits of surgical resection as long as it is estimated that at least a tenfold reduction in tumor bulk has been achieved. Indeed, enlarged mesenteric lymph nodes draining regions of lymphomatous bowel frequently prove to contain no detectable tumor and exhibit reactive hyperplasia on histologic examination.

The rationale for surgical resection of tumor is based upon observations in both African and American patients in whom tumor has been resected in the above circumstances.[77,81] Such patients, if treated almost immediately with chemotherapy, enjoy an excellent prognosis (in excess of 90% long-term, disease-free survival); removal of tumor bulk will also lessen or completely avoid the renal and metabolic complications associated with chemotherapeutic remission induction in patients with large tumor burden. In many patients in the United States, total resection is accomplished at initial surgery, undertaken because of bowel involvement giving rise to obstruction (especially intussusception), perforation, hemorrhage, symptoms of appendicitis, or a palpable mass.[58]

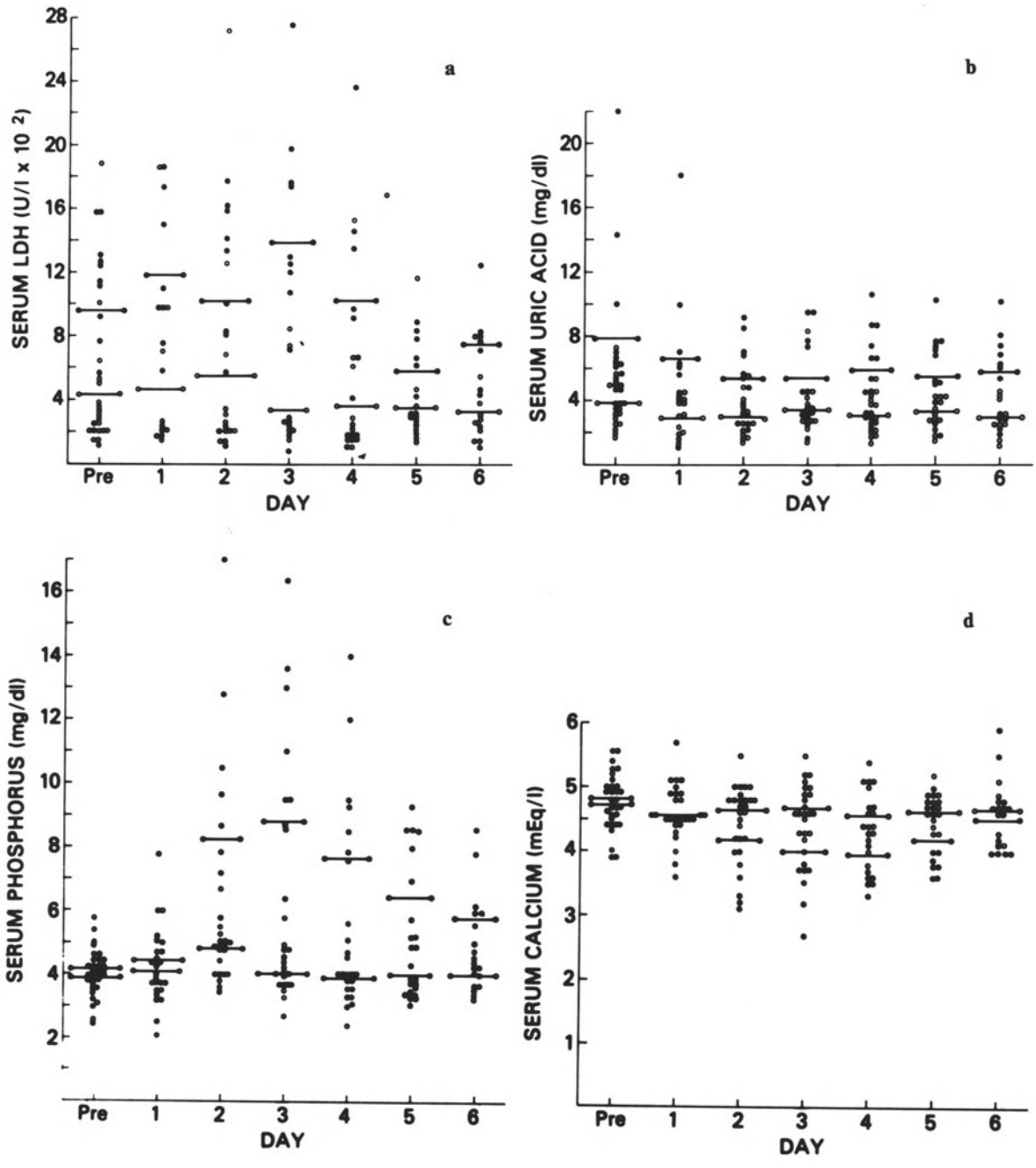


FIGURE 7–8 (a–d) Time course of biochemical changes during treatment in 33 patients with non-Hodgkin's (predominantly Burkitt's) lymphoma. **(a)** Serum LDH. **(b)** Serum uric acid. **(c)** Serum phosphorus. **(d)** Serum calcium. ●—azotemic patients; ○—non-azotemic patients (From Tsokos et al.,[130] with permission.)

The benefits of surgical resection, however, are lost in this rapidly growing tumor if chemotherapy is not begun within days of surgery. In Africa, cyclophosphamide has been administered during surgery. In the United States it is more usual to await recovery of the surgical ileus and begin treatment within 3–5 days.

Although it is possible that patients in whom all tumor can be resected represent a biologically distinct patient group (e.g., one with a smaller tumor burden) which would enjoy an excellent prognosis even without surgical resection, this seems unlikely. In Africa, for example, removal of 1 of 2 involved ovaries does not improve progno-

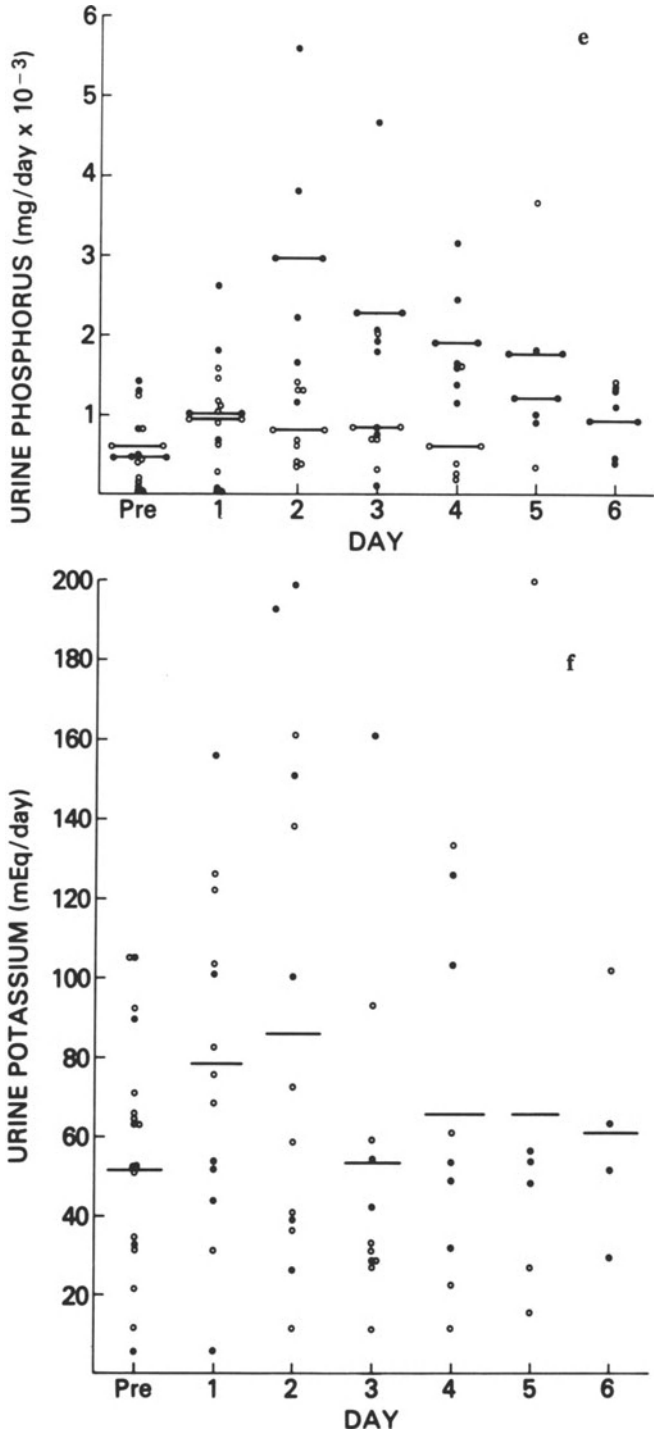


FIGURE 7–8 (e–f) Time course of biochemical changes in 33 patients with non-Hodgkin's (predominantly Burkitt's) lymphoma. (e) Urine phosphorus. (f) Urine potassium. ●—azotemic patients; ○—non-azotemic patients. (From Tsokos et al.,[130] with permission.)

sis compared to similar patients in whom no tumor is resected.[81] Patients in whom both involved ovaries are removed, however, accomplishing a tenfold reduction in tumor bulk, enjoy an excellent prognosis. In the United States, although numbers are small, even patients in whom large quantities of tumor are removed can enjoy an excellent prognosis, suggesting that the outcome of treatment is dependent upon the tumor burden after surgery rather than that before surgery.

One caveat is that as the results of chemotherapy improve, the benefits of surgery may become

less apparent. Nonetheless, if surgery is performed because of bowel perforation or obstruction, removal of all tumor may in any case be the surgical treatment of choice; and until patients with extensive, unresected intra-abdominal disease have as good a prognosis as patients with completely resected disease, it is prudent to continue to resect all tumor where possible.

Radiotherapy

In BL, the efficacy of radiotherapy has still not been well defined. An early study in Africa demonstrated responses, but these were usually partial—only 1 of 9 tumors regressed completely with doses up to 5000 rads (mean dose 3100 rads over 20 days).[97] Multiple fractions of radiation in a single day appeared to be much more effective. Of 34 tumors treated with 27 fractions to a mean dose of 2800 rads over 13 days, 25 achieved complete remission.[98] However, radiotherapy alone is not effective therapy even in patients with apparently localized disease, because of the high risk of recurrence at other sites.[47] Radiotherapy has been directed to sites of bulk disease in North American patients also receiving chemotherapy.[29,60] When combined with modern multiple-drug therapy, however, radiotherapy has not been demonstrated to be advantageous. In fact, in one study of childhood non-Hodgkin's lymphoma, which included patients with BL, radiotherapy increased toxicity and did not improve prognosis.[92]

In patients treated at the NCI, whole abdominal irradiation (2100 rads midline dose) was given in patients with abdominal tumors in some protocols. Although direct comparison of results with and without radiotherapy in patients treated with the same chemotherapy has not been made, no obvious benefits were observed, and 60% of relapses in these patients occurred in the abdomen during or shortly after radiotherapy.[143,144] The results of a more recent protocol (see below) not incorporating radiotherapy are certainly no worse, and intra-abdominal relapse is in fact less common in the later protocol. Thus available data do not support a role for radiotherapy. A small, retrospective study has suggested, however, that patients with limited, completely resected, intra-abdominal disease can achieve prolonged survival after whole abdominal irradiation.[61] This suggestion has never been confirmed prospectively.

Although considered to be standard therapy for CNS disease, including spinal cord compression, in many tumors, there is no evidence in BL that radiotherapy improves either the rapidity or the completeness of response when compared to chemotherapy alone. In a surprisingly high fraction of both American and African patients with CNS disease, a complete, durable response has been achieved with chemotherapy alone. The potential toxicity of CNS irradiation combined with chemotherapy, both to the nervous system (leucoencephalopathy) and to the bone marrow (particularly when craniospinal irradiation is used), dictates that such combinations should not be indiscriminately used in the absence of the demonstration of the superiority of such combined modality therapy over chemotherapy alone. If radiotherapy is used, it should probably be limited to a total dose of 1400 rads to the brain or an epidural tumor. In exceptional circumstances (e.g., intracerebral disease), however, radiotherapy may be the most effective therapy available. Further studies will be needed to define its role in other situations; it must be remembered, however, that there is an increased potential for local as well as general toxicity when radiotherapy is combined with doxorubicin and possibly methotrexate (skin erythema, wound breakdown, stomatitis).

Chemotherapy

Because of the relatively rare occurrence of localized disease, and the high risk that even in this circumstance relapse at other sites may occur, chemotherapy is considered to be the primary therapeutic modality in BL. Probably because of its very rapid proliferative rate, and possibly also because of the biologic nature of the disease,[76,77] BL is one of the most highly responsive of all tumors to chemotherapy. The malignant clone appears to occupy only a narrow differentiation range and is thus very homogeneous compared to tumors of mature cell types, such as nodular lymphomas or myeloma; in the latter tumors the malignant clone may extend through several differentiation steps and is therefore heterogeneous with regard to cell type. These different cell types may have differing sensitivities to chemotherapy.

In Table 7–10 is a list of agents known to be "active" in African BL. Many of these drugs are highly effective and in African patients have even induced long-term disease-free remissions when used alone. In American patients there is little information on the efficacy of single agents in BL, but a small number of patients, all of whom had

TABLE 7–10 Single-Agent Activity in African Burkitt's Lymphoma (Prior to 1970)

Drug[a]	Patients	Complete Responses	Complete + Partial Responses	Percentage Responses
Cyclophosphamide	163	43	132	81
Nitrogen mustard	61	10	44	72
Melphelan	26	8	16	61
Chlorambucil	12	3	10	83
Procarbazine	6	0	0	0
Orthomerphalan	14	?	14	100
BCNU	5	0	4	80
Vincristine	21	10	17	81
Vinblastine	2	0	0	—
Methotrexate	45	11	26	58
6-Mercaptopurine	3	0	0	—
Cytosine arabinoside	3	2	2	—
Epipodophyllotoxin	2	2	2	—
Actinomycin D	4	1	4	—
Terephthalanilide	18	1	14	78

[a] A variety of doses and regimens were used.
Data largely compiled from refs. 28, 31, and 32.

a small tumor burden, have achieved prolonged survival when treated with cyclophosphamide alone[8] (see below). More recently, because of the good results obtained with combinations of drugs, single-agent therapy as primary treatment has been abandoned.

One difficult in evaluating the literature on BL outside Africa is that only in recent years has it been widely accepted that BL constitutes a significant fraction of childhood non-Hodgkin's lymphomas (between one-third and one-half). Thus, in earlier studies, BL is not clearly distinguished from lymphoblastic lymphoma and large cell lymphomas. Nonetheless, it can be reasonably assumed that a high proportion of abdominal lymphomas in children would today be diagnosed as BL, or one of its synonyms, by most pathologists. Thus, there is no doubt that prior to 1975 the results of treatment of BL, even though not recognized as such and included among patients with "non-Hodgkins lymphoma," were extremely poor (Table 7–11).[10,20,59,71,111,117] Since that time, a number of different combination chemotherapy regimens have been shown to be effective, giving overall

TABLE 7–11 Results of Treatment of Childhood[a] Non-Hodgkin's Lymphoma Prior to 1975: Representative Series

Authors		Study Period	No. of Patients	Median Survival (months)	Survival at 5 Years[b] (%)
Rosenberg et al.[117]	Memorial	1928–1958	69	7	17.4
Bailey et al.[10]	Mayo Clinic	1945–1954	48	5	21
Sagerman et al.[120]	Columbia	1952–1964	30	6	3
Sullivan[129]	M.D. Anderson	Prior to 1967	51	6	11
Landberg et al.[70]	Lund	1944–1972	27	4	4
Jenkin[59]	Toronto	1958–1971	99	7	15
Lemerle et al.[71]	Villejuif	1950–1972	190	6	25
Pinkel et al.[111]	St. Jude's	1962–1973	64	10	30
Wollner et al.[132]	Memorial	1964–1971	43	7	11
		1966–1971[c]	18	12	33

[a] Age less than 19 years, most series less than 15 years.
[b] Actual 5-year survival or actuarially predicted 5-year survival.
[c] Treated according to the lymphosarcoma protocol.

results in the range of 40%–70% long-term survival.[62,92,109,136,143,144] Because of the rarity of the disease, many of these series are small in size, and differences in the results may therefore reflect inhomogeneity in the respective series rather than the efficacy of the particular chemotherapeutic regimen.

Perhaps the single most effective drug in BL is cyclophosphamide. This has for many years been a component of almost all effective chemotherapy regimens in both Africa and the United States. Cyclophosphamide is most often used in combination with vincristine, methotrexate, prednisone, and frequently an anthracycline. Some of the more effective combination chemotherapy regimens are shown in Table 7–12.

Design of Chemotherapy Regimens

BL is highly responsive to a number of chemotherapeutic agents, but cure is still not achieved in the majority of patients with extensive disease. This tumor thus provides an excellent model in which to learn the principles of chemotherapy in man—at least as they apply to high growth fraction tumors. Unfortunately, in spite of numerous attempts to rationalize combination chemotherapy regimens, the design of drug protocols remains largely empirical. Most regimens have adopted intermittent pulses of treatment rather than low-dose continuous therapy based on animal studies with alkylating agents and some early (though perhaps not definitive) work in Africa indicating an advantage of single-dose, intravenous cyclophosphamide over low-dose, continuous oral cyclophosphamide.[32] The optimal intensity of the pulses and their most effective composition (i.e., number and dose of drugs) is still a topic for research. There are several considerations in this regard. Very high dose chemotherapy with cell-cycle nonspecific agents is likely to cause a greater fractional cell kill, based on the work of Skipper and Schabel in animal models.[126] However, this benefit could be eroded if marrow recovery is delayed, permitting clinical tumor regrowth before marrow recovery; this phenomenon has been observed, and thus is not a purely theoretical consideration. The intensity of the pulse will impose, by virtue of myelosuppression, a lower limit to the intervals between treatments unless non-marrow-toxic, or minimally marrow-toxic drugs are interspersed between highly myelosuppressive pulses. In the current NCI protocol a 42-hour methotrexate infusion is delivered at or close to the nadir of chemotherapy-induced myelosuppression in an attempt to destroy more tumor cells at a time when they may be more sensitive than marrow stem cells.

The composition of the pulses must still be decided on a largely empirical basis. Much higher doses of cyclophosphamide than are currently employed in most regimens can be safely given, even without marrow reconstitution when adequate supportive care can be delivered.[5,6] It is not known, however, whether high doses of cyclophosphamide, or another highly effective single agent,

TABLE 7–12 Effective Chemotherapy Regimens for Burkitt's Lymphoma

Authors	Drug Combination[a]	No. of Cycles or Duration of Therapy	Radiotherapy	Predicted 3-Year Relapse-Free Survival (%)
Olweny et al.[101,103]	CTX, MTX, VCR	2	None	>50
Nkrumah et al.[96]	CTX, Ara-C, VCR	3	None	41[b]
Ziegler[136]	CTX, MTX, VCR	3	Abdomen	52
Ziegler et al.[136,144]	CTX, MTX, VCR, Pred	3	Abdomen	54
Philip et al.[109]	CTX, DXR, VCR, Pred	12–30 mo[c]	Primary site, cranial vault	40
Jenkin et al.[62]	CTX, VCR, Pred, MTX	18 mo	>3 cm masses, cranial vault	56[d]
Magrath et al.[86]	CTX, DXR, VCR, Pred, MTX	15	None	60

[a] All regimens included intrathecal drugs. CTX, cyclophosphamide; MTX, methotrexate; VCR, vincristine; Ara-C, cytosine arabinoside; Pred, prednisone; DXR, doxorubicin.
[b] Includes only patients with intra-abdominal tumor.
[c] Duration dependent upon stage.
[d] Includes all childhood lymphomas other than lymphoblastic or patients with localized disease.

would be preferable to a combination of drugs. Although there are theoretical advantages that favor the latter, there is no direct evidence that speaks to this. Theories such as the Norton-Simon hypothesis[99] or the Goldie-Coldman hypothesis,[48] while of considerable interest and sometimes quoted to justify specific drug schedules (often in retrospect!), have not been directly tested in either animal models or man. In practice, drug regimens are designed as modifications of successful approaches or combinations of several effective approaches.

In recent years there has been a trend to increase the number of drugs used in combination; while this may decrease the likelihood of drug resistance (or of combating small numbers of inherently resistant cells), it can also be disadvantageous if drugs differ in efficacy, particularly where sequential administration of different agents is employed. Too long an exposure to less effective or ineffective drugs may permit tumor regrowth, and increase, ultimately, the likelihood of the emergence of cells resistant to previously effective drugs. This may be the reason that the LSA_2-L_2 protocol containing 10 different drugs is not effective in BL.[62] Nonetheless, the evidence supports the notion that combinations of 3–5 drugs are more effective than cyclophosphamide alone, at least in currently used doses of the latter agent.

Finally, the duration of therapy has also not been well established. Some regimens employ 2–3 years of therapy[91,92,132] in spite of the lack of evidence that there is any advantage to maintenance therapy. African patients with small tumor burdens have achieved long-term survival after a single dose of cyclophosphamide,[25,28,32] and the results of regimens in the United States using only three cycles of combination therapy have been surprisingly good[136,143,144] (see below). It is likely, based on reported studies, that in the future chemotherapy will be continued for 6 months to, at most, 1 year, the precise duration perhaps depending upon disease extent and rapidity of remission induction.

It should be borne in mind that tumor burden appears to be the single most important prognostic indicator in BL,[84] and it is entirely logical to tailor both intensity and duration of therapy to tumor burden. This has been done to some extent already and perhaps will increasingly become a feature of therapy in the future. It becomes particularly important to reduce the potential hazards of chemotherapy to a minimum in patients with an excellent chance of long-term survival.

Prophylaxis Against Central Nervous System Recurrence

Regardless of the effectiveness of systemically administered agents, since CNS recurrence is such a common feature of BL throughout the world, a high failure rate must be expected if an attempt is not made to prevent CNS spread. Remarkably (compared to other neoplasms), CNS involvement in BL can be cured—sometimes with only a small number of intrathecal injections of cytosine arabinoside, methotrexate, or both drugs.[121,145] Thus, the intrathecal injections of either or both of these agents ought to effectively prevent the development of overt CNS disease. Although early trials carried out in Uganda did not show any benefit of such prophylactic therapy,[137] there was such a high rate of systemic recurrence in the patients included in these trials that it must be deduced that systemic treatment was relatively inefficient. CNS recurrence could therefore have resulted from spread from systemic tumor (possibly subclinical) remaining after completion of CNS prophylaxis, and does not necessarily indicate failure of this approach to CNS prophylaxis. A similar argument may be made with regard to a randomized trial, in Africa, of craniospinal irradiation after remission induction, which failed to show a benefit of radiotherapy. CNS relapse in this trial was, however, frequently the sole initial site of relapse whether or not irradiation had been given.[102] Some evidence for the value of intrathecal prophylaxis is provided by combination chemotherapy trials that incorporated such a component; in these trials only a low frequency of CNS recurrence was observed.[96,103] At the NCI, the introduction of an intensive intrathecal prophylactic regimen into the current protocol resulted in a lower frequency of CNS recurrence in patients who did not have CNS disease at presentation.

An alternative approach to prophylaxis with intrathecal drugs is the use of agents that cross the blood–brain barrier. These have the advantage of even distribution throughout the CNS, and more certain entry into the CNS than intrathecal drugs. A randomized trial of relatively low-dose CCNU in Uganda was, however, ineffective (in fact, patients treated with CCNU had an overall worse prognosis),[142] and 42-hour methotrexate infusions without intrathecal drugs permitted 4 CNS

recurrences in 13 patients with BL in the United States.[122] However, failure of these particular regimens does not imply that this general strategem of systemically administered drugs for CNS prophylaxis is invalid. Higher doses, or a different interval between drug administrations, may be necessary. Timing of prophylactic therapy may be critical, and we have favored the commencement of this treatment simultaneously with initial chemotherapy. However, no data exist to speak to this point.

Treatment of Overt Central Nervous System Disease
Chemotherapy has been the primary modality of treatment for overt CNS disease in Africa, including meningeal involvement, cranial nerve palsies, and epidural tumor.[77,103,135,137,138,145] Tumor at the latter site is rare in the United States, and almost all patients in Africa with spinal cord compression have been treated with chemotherapy alone since radiotherapy is not generally available in Africa. Dramatic responses have occurred after a single dose of cyclophosphamide, with rapid and permanent recovery to normal or almost normal neurologic function. Some patients, however, have been rendered permanently paraplegic, probably because of myelonecrosis secondary to impairment of the blood supply of the spinal cord.[133] Although no comparative data are available, the rapidity of response in some patients suggests that irradiation would add nothing to chemotherapy. Where myelonecrosis has occurred, there is not likely to be a response of the paraplegia to any therapy, although long-term survival may be achieved.

Cranial nerve palsies and meningeal involvement have been treated in Africa with intrathecal methotrexate and/or cytosine arabinoside combined with systemic therapy.[137] A significant fraction of African patients with CNS involvement achieve prolonged survival when so treated. Furthermore, approximately 50% of all long-term survivors have had CNS disease at some time (presentation or relapse), indicating the success of chemotherapy alone in eradicating CNS disease.[145] Similarly, in the United States a small number of long-term survivors who had CNS disease at presentation never received radiotherapy.[122] Patients who relapse with CNS disease, or patients with CNS disease presenting at centers other than the NCI, are usually treated with cranial irradiation and intrathecal therapy, but data are inadequate to assess the additional value, if any, conferred by CNS irradiation. The additional degree of myelosuppression, particularly if craniospinal irradiation is used, must be taken into consideration, for this may seriously impair the ability to deliver systemic therapy, and may therefore actually be detrimental to survival. Thus, at present, at the NCI cranial irradiation is reserved for patients who have failed intrathecal therapy. In one such patient, long-term survival was achieved after two episodes of CNS involvement: the first, at presentation, was treated with intrathecal cytosine arabinoside and methotrexate; the second was treated with the same drugs and cranial irradiation.[122]

The role of drug delivery via Ommaya reservoir, while theoretically attractive, has also not been directly compared to drug delivery by lumbar puncture in terms of its effectiveness in treating CNS disease; in many centers this form of therapy is reserved for patients with recurrent CNS disease.

The use of high intravenous doses of drugs that cross the blood–brain barrier in significant amount (nitrosoureas, methotrexate or cytosine arabinoside) is worthy of further exploration, since this may achieve the most uniform delivery of drug to the CNS. We have observed clearance of malignant pleocytosis following 42-hour methotrexate infusions that achieve mean levels of close to 10^{-6} M methotrexate in the cerebrospinal fluid, but more work needs to be done in this area.

Results of Treatment

African BL

Following the initial, very promising results of single-agent chemotherapy therapy in African BL at a time when very little evidence existed for a more than temporary value of chemotherapeutic agents,[25,28,31,32,139] there has been a gradual evolution to multiple drug therapy. In Uganda, a series of randomized chemotherapeutic trials were carried out to determine the following: whether 6 doses of cyclophosphamide were better than 1[103,138,139]; whether a 4-drug, sequential combination—cyclophosphamide, vincristine, methotrexate, and cytosine arabinoside (TRIKE)—was better than multiple doses of cyclophosphamide in patients with extensive disease[103,135,141]; and, more recently, whether a simultaneous combination of cyclophosphamide, vincristine, and methotrexate

(COM) was better than cyclophosphamide alone.[101,103,135,138,139,141] These trials are difficult to interpret because of the small numbers and the relatively high frequency of CNS relapse. (CNS prophylaxis was, in general, not given because of the results of a previous small study that did not indicate an advantage of intrathecal therapy.) Furthermore, relapse in African BL, whether in the CNS or systemic, is frequently compatible with prolonged survival.[145] This means that comparisons of survival may not be fair tests of the initial therapy. There is, however, an indication that patients with extensive disease had longer remission durations when treated with TRIKE in comparison to 6 doses of cyclophosphamide, and a significantly improved survival was observed in patients treated initially with TRIKE or COM compared to patients treated with cyclophosphamide alone (Table 7–13).[103] Taken together, about 80% of Ugandan patients achieved complete remission, and of these patients 50% achieved prolonged survival. The overall survival in 280 patients with sufficient staging and follow-up information was 39%.[103]

The apparent advantage of drug combinations in Ugandan patients is consistent with the results of a retrospective comparison of a combination of cyclophosphamide, vincristine, cytosine arabinoside, and intrathecal methotrexate with cyclophosphamide alone, carried out in Ghana in patients with abdominal involvement.[96] Although the combination regimen was more toxic and led to more early deaths, such that no ultimate survival advantage was demonstrated, the patients who achieved remission with the combination regimen had a significantly lower chance of relapse, whether in the CNS or systemically. With the combination regimen, 80% of patients with extensive disease who achieved complete remission remained free of CNS recurrence, while about half that number treated with cyclophosphamide alone did so. Overall relapse rates in complete responders were 31% for patients treated with the combination and 62% for those given cyclophosphamide alone. The reason for the advantage in remission maintenance of the drug combination is debatable. It could as well be due to effective CNS prophylaxis as to the combination of systemically effective drugs. This is of interest, since in the Ugandan trials, neither intrathecal drugs nor craniospinal irradiation could be shown to provide effective prophylaxis against CNS relapse.

In all the African trials, the prognostic significance of stage—probably a reflection of tumor burden—was clearly demonstrated.

North American Burkitt's Lymphoma

In considering the results of treatment of BL outside Africa, one important issue cannot be avoided: the definition of the disease. As has been discussed

TABLE 7–13 Treatment Protocols and Survival in Ugandan Patients with Burkitt's Lymphoma

Patients Studied	Treatment[a]	AFD[b]	Dead	LTFU[c]	Total
All	Cyclophosphamide 1 dose	9	6	0	15
vs	Cyclophosphamide 6 doses	9	6	1	16
III[d] and IV[d]	Cyclophosphamide 6 doses	1	8	1	10
vs	TRIKE	14	6	0	20
All	BCG	13	10	1	24
vs	No BCG	10	6	2	18
All	Cyclophosphamide 2 doses ±CSIR	4	17	1	22
vs	COM 2 doses ±CSIR	15	13	2	30
All	COM ± i.t. MTX	11	3	2	16
	Nonprotocol patients	23	47	6	76
	Early deaths	—	33	—	33
Total treated		109	155	16	280

Adapted from Olweny et al.[103]

[a] TRIKE, cyclosphosphamide, vencristine, methotrexate, and cytosine arabinoside; BCG, bacillus Calmette-Guerin vaccine; CSIR, craniospinal irradiation; COM, cyclophosphamide, vincristine, and methotrexate (Olweny et al.[101]); i.t., intrathecal; MTX, methotrexate.

[b] Alive and free of disease.

[c] Lost to follow-up.

[d] Old staging (Ziegler et al.[139]).

earlier, the diagnosis is currently a histologic one. In spite of the publication, however, of a "definition" of BL, histopathology is, by nature, a subjective discipline. Thus, there must always remain cases in which not all pathologists, however expert, would agree over the diagnosis. Furthermore, within the category of malignant lymphoma of small, non-cleaved cells defined by Lukes, which corresponds to Rappaport's "undifferentiated lymphoma," some would maintain a division into Burkitt's and "non-Burkitt's" lymphoma, whereas others believe that relatively small histologic differences do not indicate the existence of separate pathologic entities.[77] Careful studies between centers to examine whether the distinction can be made reproducibly have not been done, although the results of a large histologic review by many pathologists revealed considerable variation.[95]

While this debate remains unresolved, most clinicians would agree that if differences in anatomic location of tumor or response to therapy exist between Burkitt's and non-Burkitt's undifferentiated lymphoma, at least in children, then they are extremely subtle and difficult to detect. Thus, for the purposes of this discussion, the results obtained by centers where the distinction is not made will be included. The limited data concerning differences or similarities in therapeutic responses will, however, also be presented. A further note of caution is appropriate with regard to the reproducibility of the histologic distinction between the large cell lymphomas of childhood and undifferentiated lymphomas. Although some tumors can be reproducibly categorized as one or other, there is also overlap between the histologic appearances. Once again, no data have been collected that correlate therapeutic outcome with histology, but it must be borne in mind that the stated histologic compositions of different series are only "semiquantitative" and could well differ considerably if reviewed by different pathologists or even rereviewed by the same pathologists. One unifying feature of these three histologic entities is that all are the neoplastic counterparts of cells in the B lymphocyte differentiation series in that they express surface immunoglobulin, essentially always IgM.[76] The tumors under discussion here have also been shown to be monoclonal (where tested), i.e., express only a single light chain class (PGMP),[88] as opposed, for example, to immunoblastic lymphomas associated with immunodeficiency syndromes, which are frequently polyclonal.[78]

National Cancer Institute Studies

At the NCI there has been a longstanding interest in BL. Initially, treatment was not according to a standard protocol, and it is interesting to note that, prior to 1974, 5 patients, all of whom had intra-abdominal tumor, were treated with radiotherapy alone.[8] Four of these patients had ileal or ileocecal masses and may have today enjoyed prolonged survival with a combination of surgery and chemotherapy. Three of the 5 patients had no response, and two had incomplete responses. All were dead within 2 months of diagnosis. Two other patients were treated with either the nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP) or POMP combination chemotherapy regimens and died after 6 months and 1 month, respectively, never achieving complete remission.

The remaining 22 patients reported[8] were treated with cyclophosphamide alone (40 or 75 mg/kg i.v.), either 1 or 6 doses; 13 achieved complete remission. All except 1 had been treated with 6 doses of cyclophosphamide; the exceptional patient had oral cyclophosphamide after a single intravenous dose. Of the patients who did not achieve complete remission, 4 died abruptly and so had received only a single dose of cyclophosphamide. Of the 13 complete responders, 9 had survived for between 28 and 81 months and 1 for 6 months at the time of writing; 4 patients had relapsed, 3 of whom had died. Three of the long-term survivors had stage I or II disease [cervical adenopathy only (2 patients) or cervical adenopathy and maxillary tumor (1 patient)] and 5 of the other 6 appear to have had abdominal tumor completely resected. Thus, almost all the patients who achieved prolonged survival following cyclophosphamide as the sole chemotherapy had had small tumor burdens.

Commencing in 1974, patients at the Pediatric Oncology Branch of the NCI were treated with a combination of cyclophosphamide, vincristine, and methotrexate with prednisone (COMP)[144] or without prednisone (COM)[141] (Table 7–12). Patients with intra-abdominal disease (with a small number of exceptions) received whole abdominal irradiation (2100 rads over 18 days).

Of the 37 patients treated with either COM or COMP, 18 achieved prolonged relapse-free survival, and another 4 patients achieved prolonged disease-free survival after treatment of relapse; 2 of the latter patients had isolated CNS relapse,

and 2 others were treated with the very intensive BACT regimen (see below). The results of treatment of patients in other centers with the same initial regimens were essentially identical.[136] Although the overall survival rate of these combination regimens does not differ significantly from that obtained with cyclophosphamide alone (9 of 22 patients),[8] 11 of the long-term survivors treated with COM or COMP (including 7 who never relapsed) had stage D disease (see Table 7–4).

These regimens are of considerable interest, for the results were achieved with only 3 cycles of therapy. The role of intra-abdominal irradiation is not clear from these data, as discussed above, but in 7 patients of 10 with intra-abdominal recurrence, relapse occurred during or shortly after the completion of radiotherapy, indicating that radiotherapy probably did not improve the outcome. The remaining 3 patients had not received abdominal irradiation.

Subsequent to the COM and COMP protocols, a much more intensive protocol was introduced in an attempt to improve significantly upon overall survival rate.[77,86] A major consideration in its design was to try to prevent regrowth of tumor between chemotherapy cycles. Basically, the protocol consists of a 4-drug combination (cyclophosphamide, vincristine, prednisone (COMP), adviamycin) alternating with an infusion of methotrexate given at the nadir of myelosuppression. It was reasoned that an S-phase agent should be effective at destroying regrowing cells but should have much less of an effect on an already suppressed bone marrow than other agents (e.g., alkylating agents) known to be effective against BL. Furthermore, methotrexate would be an additional effective agent which would be given as a constant infusion for as long a period as deemed tolerable. Doxorubicin (Adriamycin) was added to the 4 other drugs used in previous protocols because of its broad range of activity and promising early results from Africa in a few patients treated with a combination of doxorubicin and cyclophosphamide. It was decided to continue therapy for approximately 1 year since the potential benefit of more prolonged treatment had not been explored, although in several centers in the United States patients were being treated with leukemia-like maintenance regimens (i.e., methotrexate and 6-mercaptopurine for 2–3 years)[92,132] (see below). Because of the presumption that patients with limited or completely resected abdominal tumor would have a very good prognosis even without prolonged therapy, such patients were treated in protocol 77–04 with only 6 cycles of therapy.

The results in 46 patients with BL, from a group of 54 patients with undifferentiated lymphoma, are shown in Figure 7–9.

The best results were clearly achieved in the group of patients in whom all intra-abdominal tumor had been resected prior to chemotherapy (stage AR), even though such patients had only six cycles of therapy. Although some of these patients had minimal disease (e.g., as little as 1 cm^3 of tumor in the small bowel, sufficient to cause intussusception), others had very extensive disease (e.g., bilateral massive ovarian enlargement). Only 1 of 15 patients with resected disease has died, and he had extensive retroperitoneal disease extending into the thigh—probably not in the same category, either in terms of tumor burden or fraction of tumor resected, as the other patients.

Of the 54 patients with undifferentiated lymphoma treated, 7 failed to achieve complete remission. All were 16 years old or more. Seven patients had a first relapse in the CNS; 4 of these 7 patients were included among the first 13 patients treated on protocol 77–04 and had never received intrathecal prophylaxis. Because of this early high rate of CNS recurrence, an intensive intrathecal regimen (modeled on the intrathecal regimens used successfully for the treatment of overt CNS disease) was introduced into protocol 77–04. One patient has since failed intrathecal prophylaxis, and 2 both presented and recurred with CNS involvement. Seven patients relapsed systemically, 2 in the bone marrow, 2 in the spermatic cord, 1 in the testis, and 2 in the abdomen (1 of the latter 2 was accompanied by bone marrow and CNS involvement). Ultimately, nearly all the patients who died had bone marrow involvement.

The relapse pattern is rather different from that seen in the previous protocols (COM and COMP), in which a much higher proportion of patients had systemic relapse, especially in the abdomen and bone marrow (Table 7–14). It is not clear why there appear to be rather more partial responders in the current protocol. The possibility that this is because of more rigorous methods (CAT scan and ultrasound) of documenting complete remission in the current protocol, such that some patients who would have been called complete responders previously are now included as partial responders, must be considered; however, this is

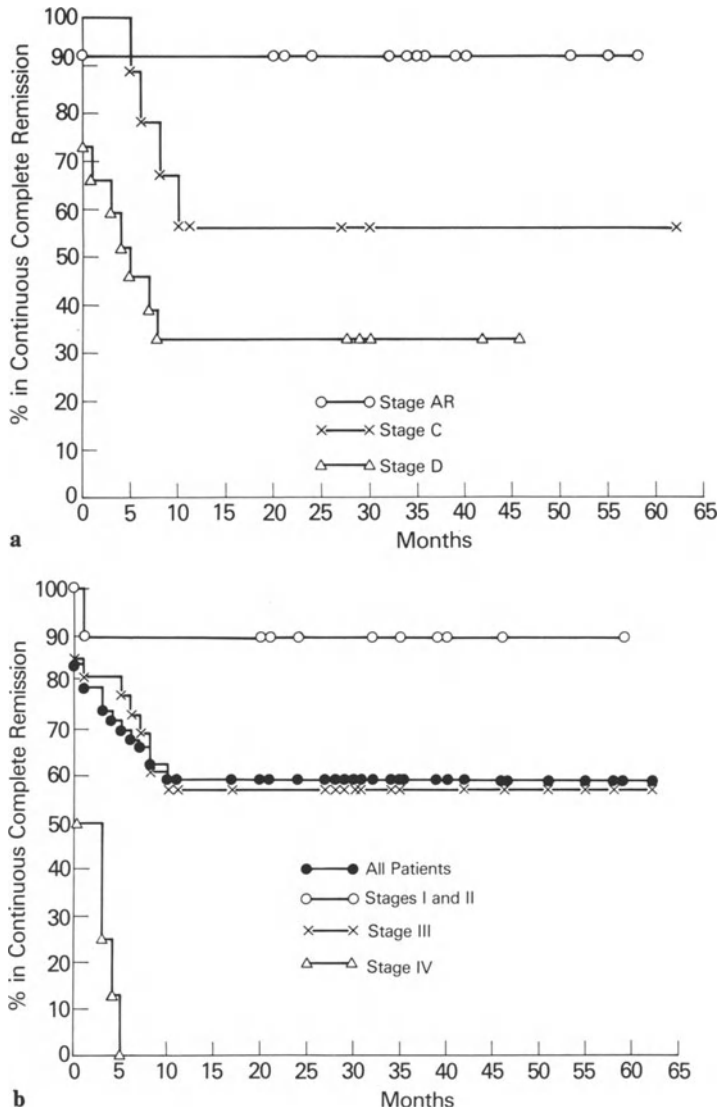


FIGURE 7–9 Results of treatment of Burkitt's lymphoma with NCI protocol 77 04. **(a)** Kaplan-Meier plots of patients in stages AR, C, or D (see Table 7–4) in continuous complete remission. Stages A and B are not shown since numbers in these stages are so small. **(b)** Kaplan-Meier plots of patients in Murphy's stages I and II combined, III, and IV, and the entire group in continuous complete remission. It is important to note that stage IV in this series includes patients with diffuse (>50%) bone marrow infiltration, unlike in other series, in which patients with more than 25% marrow infiltration are usually excluded.

not a complete explanation since most of the partial responders in protocol 77–04 were apparent from clinical examination. An alternative explanation is the greater median age of 77–04 patients. In a larger study of 54 patients (including non-NIH patients) treated by the COM or COMP protocols, there were 2 partial responders, so that the difference is, in any event, not statistically significant.

Since 4 of the 7 CNS relapses in protocol 77–04 occurred in patients not given intrathecal CNS prophylaxis, and, in fact, only 1 patient failed CNS intrathecal prophylactic therapy, it is unlikely that CNS relapse will constitute a major problem in the future as long as intensive intrathecal prophylaxis is given. It is of interest that abdominal recurrence occurred in 9 of 26 (33%) patients who had had abdominal irradiation in the COM and COMP protocols (excluding partial responses), but in only 4 of 54 patients (7%) in 77–04, in which abdominal irradiation was not given. This difference is statistically significant ($p = 0.007$). Among 48 patients with intra-abdominal disease treated on protocol 77–04, only 10 patients (20%) failed in the abdomen, including 6 partial responders. This strongly suggests that radiotherapy (or at least abdominal irradiation) is not a useful treatment modality for abdominal lymphomas treated intensively with chemotherapy.

Whether the current protocol has any advantage over the previous protocols is not entirely clear since numbers for comparison are quite

TABLE 7–14 Reasons for Failure in National Cancer Institute

Outcome	Protocol	
	COM/COMP	77–04
Total patients	37	54
Partial response	0	7 (13)[a]
Isolated CNS relapse	2 (5)	7 (13)
Systemic relapse[b]	15 (40)	7 (13)
Bone marrow relapse	7 (19)	2 (4)
Total failures	17 (45)	24 (39)

[a] Percentage of total patients is indicated in parentheses.
[b] Includes bone marrow.

small, and some differences exist between the two series of patients (e.g., in median age). At the present time, however, there is a trend that favors protocol 77–04 with regard to the fraction of patients in complete remission who remain free of relapse. In the COM and COMP protocols, 18 of 35 patients (51%) who achieved complete remission never relapsed, whereas in 77–04, 33 of 47 patients who achieved complete remission (70%) remain in continuous complete remission. Emphasis in the future will need to be on patients with chemotherapy-resistant tumors from the outset and those with extremely widespread disease.

Children's Cancer Group Study and Other Studies from the United States and Canada

One large North American clinical trial that includes BL is that of the Children's Cancer Study Group (CCSG).[61] In 1977 the CCSG initiated a randomized trial in children below 17 years old with non-Hodgkin's lymphoma and less than 25% lymphoma cells in the bone marrow. (This criterion was not used in the NCI trials and should be borne in mind when making comparisons. In protocol 77–04, for example, 8 patients had diffuse bone marrow involvement of over 50%, and only one remains alive. Exclusion of these patients would bring the proportion that achieved continuous complete remission to over 70%.) Patients were randomized to either a 4-drug (cyclophosphamide, vincristine, methotrexate, and prednisone) arm (Table 7–12), or a ten-drug arm—a slightly modified version of the LSA_2-L_2 regimen described by Wollner et al., which was developed at Memorial Hospital in New York City.[132] Both arms included intrathecal methotrexate and radiation to all sites of bulk disease (over 3 cm^3). Of 235 patients entered into this study, 114 were diagnosed as having undifferentiated lymphoma after central histologic review; 45 of these 114 were diagnosed as having BL. An additional 33 patients were diagnosed as having diffuse histiocytic lymphoma.

There was a difference in relapse-free survival between the two arms of this regimen. Of those with nonlocalized, "nonlymphoblastic" lymphoma (i.e., undifferentiated and diffuse histiocytic lymphomas), 57% of 53 patients treated with the 4-drug regimen were alive and free of disease at 3 years, while 28% of 43 patients in the 10-drug arm were alive and disease free. Considering only undifferentiated non-Burkitt's lymphoma with nonlocalized disease, the respective relapse-free survival figures were 59% and 30%, while for BL the respective figures were 61% and 23%. Although the number of patients in histologic subgroups was small (32 patients with non-Burkitt's lymphoma and 23 with BL), the results of this study support the probability that histology in "nonlymphoblastic lymphomas" is not of prognostic significance. For patients with limited disease, 3-year relapse-free survival was close to 80% in all histologic groups, although, again, there appeared to be a small advantage (not statistically significant) to the 4-drug regimen. This regimen was also less toxic.

The main conclusion of this study was that the 4-drug regimen was superior to the slightly modified LSA_2-L_2 regimen for patients with "nonlymphoblastic" lymphomas. When allowance is made for the differences in entry criteria (age and bone marrow involvement) for patients entered into the CCSG study as compared to the NCI study, the results are comparable in spite of the fact that local irradiation was used, and treatment extended over 18 months in the CCSG study.

Other published series of patients with undifferentiated lymphomas treated in North America are too small to draw valid conclusions. The original LSA_2-L_2 series[132] appears to have a paucity of such conducted at St. Jude Hospital (Memphis) in patients below 19 years with non-Hodgkin's lymphoma.[92] Although 27 patients were diagnosed as having undifferentiated lymphoma, and after reclassification by the Lukes-Collins scheme, 19 were said to have small, noncleaved cell malignant lymphoma, the results of treatment were not separated from those of 20 patients classified as having lymphoblastic lymphoma (Rappaport term). Thus,

although overall disease-free survival at 2 years for the 88% of patients who achieved complete remission was predicted to be a little over 50%, and patients with limited disease had a 90% predicted long-term survival, while patients in stages III and IV (using the St. Jude system–see Murphy et al.,[92] Table 7–5) had a less than 40% disease-free survival, conclusions cannot be drawn as to the results of this protocol in patients with undifferentiated histologies. In all patients with extensive disease, however, irradiation of sites of bulk disease did not improve the therapeutic efficacy, but did increase toxicity compared to the chemotherapy regimen alone (vincristine, cyclophosphamide, doxorubicin, and prednisone followed by 2 years of maintenance with 6-mercaptopurine and methotrexate).

Because of the generally poor results of therapy in patients with extensive BL, a team at the University of Minnesota examined the role of very intensive therapy (cyclophosphamide, BCNU, cytosine arabinoside, and total-body irradiation; CRAB) followed by allogeneic bone marrow transplantation in a small group of patients with extensive disease who achieved complete response initially to standard therapy. Although 3 of 6 patients remained relapse free at the time of reporting, 2 had graft-versus-host disease.[100] Thus, the overall results of this approach may prove not to be superior to more conventional therapy.

European Studies

In a retrospective study carried out at the Centre Leon Berard, Lyon, France, in patients below 16 years of age with non-Hodgkin's lymphoma,[107] 47 of 87 patients (54%) were histologically diagnosed as having BL. These patients were treated with several regimens between 1965 and 1979, including cyclophosphamide alone, cyclophosphamide and vincristine, or, since 1973, cyclophosphamide, vincristine, prednisone, and doxorubicin (COPAD). Four of these patients died soon after presentation, and 9 (19%) had no response to therapy. The overall predicted survival at 4 years was 40%. There was a marked difference in survival between patients with limited disease (including patients with completely resected abdominal tumor), of whom more than 85% survived, and patients with extensive disease (33 patients), of whom less than 25% survived. A protocol that incorporates very high dose components is currently being used in France. The results are awaited with interest.

A study carried out in Vienna using the LSA_2-L_2 protocol[132] confirmed the findings of the CCSG group—that this protocol is considerably less effective for patients with undifferentiated histologies than for patients with lymphoblastic lymphoma (Rappaport term).[110]

Relapse Patterns and Treatment of Relapse

The primary objective of chemotherapy is to induce prolonged disease-free survival, i.e., to prevent relapse. As this goal is approached, it is to be anticipated that patients who do relapse will have highly resistant tumors and an extremely grave prognosis. In general, this applies at present to BL in the United States and Europe. At the NCI only 7 of 29 patients with BL have survived relapse: 3 had isolated CNS recurrence[122] and 4 had extensive systemic relapse, including bone marrow involvement in 3. These 4 patients achieved long-term survival after very intensive therapy[5,6] using a regimen of BCNU, cytosine arabinoside, cyclophosphamide, and 6-thioguanine (BACT) (Table 7–15). A total of 19 patients with BL were treated according to the BACT protocol, 9 of whom received autologous marrow rescue. Recovery from marrow hypoplasia appeared to be delayed by approximately 1 week in patients who did not receive a marrow autograft.[5]

The latest relapse seen at the NCI Pediatric Oncology Branch in over 100 patients with BL was at 10 months from the start of treatment. One patient with an initial diagnosis of undifferentiated non-Burkitt's lymphoma, altered to "large cell" lymphoma upon review, relapsed 3 years after

TABLE 7–15 BACT Regimen

Day 1	BCNU 200 mg/m^2
Days 2–5	Cyclophosphamide 45 mg/kg i.v. every 24 hr (4 doses, total 180 mg/kg)
	Cytosine arabinoside 100 mg/m^2 i.v. every 12 hr (8 doses, total 800 mg/m^2)
	6-Thioguanine 100 mg/m^2 i.v. every 12 hr (8 doses, total 800 mg/m^2)

presentation but has since been in complete remission for 10 months after a complete surgical resection and 6 cycles of protocol 77–04. Apart from this patient, the relapse pattern of undifferentiated non-Burkitt's lymphoma has not differed from that of BL.

It is of interest that patients who fail high-dose therapy for relapse almost invariably develop bone marrow infiltration, even though they may never have had bone marrow involvement before. Whether this is a consequence of the profound myelosuppressive effect of intensive therapy, or the development of overt marrow disease in patients with previously occult marrow involvement (which has now been documented to occur in at least 16% of patients with a microscopically normal bone marrow[127]), or even the result, in some cases, of reinfusion of bone marrow–containing occult tumor cells, is not known. It is possible, however, that the presence of bone marrow involvement, whether occult or overt, is a sign of a biologic difference that signifies a poor prognosis with current therapeutic approaches.

Irrespective of these considerations, the management of patients who relapse after intensive primary therapy or of partial responders is immensely difficult. Apart from the NCI patients who achieved long-term survival with BACT, and occasional patients treated with allogeneic or autologous marrow after very intensive chemotherapy at other centers,[63,100] there are few promising approaches. A recent pilot study at the NCI of high-dose cyclophosphamide and high-dose cytosine arabinoside with or without total-body irradiation and autologous bone marrow reinfusion resulted in only 1 long-term survivor of 15 patients treated. The results of allogeneic marrow transplantations after high-dose therapy during the first remission in patients with extensive disease do not suggest that this approach will be successful in relapsed patients. They, likewise, do not engender enthusiasm for alternative approaches to allogeneic marrow—such as autologous marrow treated with either drug[123] or antibody[114] to remove putative occult tumor cells—unless improved preparative regimens can be devised.

Future approaches include the novel use of conventional agents—e.g., high-dose cytosine arabinoside[119] or BCNU[128]—or newer methods of unknown potential—e.g., interferon, monoclonal antibodies, or even differentiation-inducing agents, such as retinoids.

In the African patient relapse patterns are in general similar, but the outcome of treatment for relapse is dramatically different. About one-half of all patients who relapse achieve prolonged survival—sometimes after a succession of relapses (up to six have been recorded prior to long-term disease-free survival—in this case, 7 years).[140,145] Many of these multiple relapses involve the CNS, and all are treated with conventional chemotherapy, often using the same drugs repeatedly. This remarkable finding has been completely unexplained. It clearly shows, however, that relapse in African BL is not simply a matter of chemotherapy resistance, although some patients do ultimately have no response to treatment. It has been stated that patients who relapse early (e.g., within 3 months) in Africa have a much worse prognosis than those who relapse late (after 3 months).[140] While this is true overall, it is also true that early relapse is much more frequently associated with patients with extensive disease. In this group a much greater proportion of relapses occurs within 3 months. If remission duration *within* stage is examined, however, there is no correlation with prognosis, so that early and late relapse seem appear to correlate directly with the initial stage of disease rather than being determinants of survival in their own right.

The peculiar relapse pattern in the African patient does raise the issue of the duration of therapy. Perhaps the relapse rate could be markedly reduced by some form of continued therapy throughout the first 6–12 months, as opposed to treatment for only 2–3 months. The value of maintenance therapy has never been studied in African BL, but it may be more appropriate in African than in American BL.

Conclusions

BL is a model neoplasm. Its provocative geographic distribution in Africa stimulated a search for a viral etiology and led to the discovery of EBV and new insights into the role of this virus in other kinds of human lymphoproliferative disease. Differences between African and American tumors have motivated studies to elucidate the cellular origins of these neoplasms. The characteristic chromosomal abnormalities in a B cell neoplasm have stimulated intensive research into pathogenetic mechanisms at a molecular level,

considerably aided by the availability of continuous cell lines. The management of this rapidly proliferative neoplasm presents a challenge to the clinician, ranging from the complications of acute tumor lysis to the development of increasingly effective drug combinations. About two-thirds of all patients can currently be cured with chemotherapy alone, or surgery followed by chemotherapy.

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CHAPTER 8

Sarcoidosis of the Lymph Nodes

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Sarcoidosis is one member of a large family of granulomatous disorders in which the common denominator is the histology of epithelioid cell granulomas. Like many other members of this loose-knit family, its etiology is unknown. It is a multisystem disorder that most commonly affects young adults presenting with intrathoracic, skin, and eye lesions. The diagnosis is most secure when clinicoradiographic findings are supported by histologic evidence of widespread noncaseating epithelioid cell granulomas in more than one system or by a positive Kveim-Siltzbach skin test. There is evidence of depression of delayed-type hypersensitivity with impairment of thymus-mediated suppressor T cells in the peripheral blood but with evidence of increased numbers and activity of helper T cells in the lungs and in bronchoalveolar fluid. These helper T cells activate B cells to produce raised serum immunoglobulins, raised lambda and kappa light chains, and increased titers of serum antibodies against many antigens. Other common features are circulating immune complexes also hypercalciuria with or without hypercalcemia.

The course and prognosis correlate with the mode of onset; an acute onset with erythema nodosum heralds a self-limiting course and spontaneous resolution, while an insidious onset may be followed by relentless progressive fibrosis (Table 8–1). Corticosteroids relieve symptoms and suppress inflammation and granuloma formation.

Pathology and Differential Diagnosis

There are numerous causes of lymph node granulomas that are histologically nonspecific (Tables 8–2 and 8–3). The diagnosis of lymph node sarcoidosis demands a synthesis of a compatible clinical picture, certain histologic features, and characteristic chest radiographic changes.

The epithelioid cell granuloma in the lymph node consists of discrete focal collections of epithelioid cells. Langhan-type multinucleate giant cells are frequently present together with admixed lymphocytes (Figure 8–1). Plasma cells are inconspicuous and eosinophils are absent. In the healing phase fibroblasts proliferate with the deposition of reticulin and production of collagen. Central necrosis is absent or inconspicuous. It is essential to exclude similar granulomas due to infections, parasitic infestation, mineral and chemical agents, and foreign material (Table 8–2).

Bone marrow and blood monocytes become activated macrophages, epithelioid and giant cells. Epithelioid cells show abundant vacuolated cytoplasm rich in Golgi complexes and smooth and rough endoplasmic reticulum. Mucoprotein-containing secretory vesicles are conspicuous, and mitochondria are well developed and prominent. Evidence of phagocytosis and lysosomes are inconspicuous. The cell margins show complex interdigitating processes, closely related to intermingled lymphocytes. Langhan-type giant cells, arising from fused epithelioid cells, show similar cytoplasmic features. The evidence suggests that these cells are secretory rather than phagocytic and that they are the probable source of angiotensin-converting enzyme and of lysozyme. The admixed lymphocytes are T cells, which are conspicuous. B cells are inconspicuous, and electron microscopy is needed to detect epithelioid cells which are rich in rough endoplasmic reticulum and probably secreting immunoglobulins.[8]

The nature of the uncommon central "necrosis" is still somewhat in dispute. It can be distin-

TABLE 8–1 Features Distinguishing Active from Inactive Sarcoidosis

Features	Active	Inactive
Age (years)	Under 35	Over 50
Onset	Abrupt	Insidious
Chest x-ray	Changing	Static
Skin	Erythema nodosum, vesicles, maculopapular rash,	Plaques, scars, keloids
Eyes	Conjunctivitis, uveitis	Claucoma, cataract
Fluorescein angiography leakage	Yes	No
Histology	Epithelioid/giant cells	Hyaline fibrosis
Calcium metabolism	Abnormal	Normal
Urinary hydroxyproline	Increased	Normal
Kveim-Siltzbach test	Positive	Negative
Tuberculin test	Negative	May be positive
T helper:suppressor ratio		
Lungs	10.5:1	1.4:1
Blood	0.8:1	1:1
I a T	Increased	Normal
B cells (activated by T helper)	Yes	No
Serum immunoglobulins	Raised	±
Kappa and lambda chains	Raised	Normal
SACE	Elevated	Normal
Serum lysozyme	Elevated	Normal
Serum collagenase	Elevated	Normal
Gallium scan	Positive	Negative
Immune complexes	Present	Absent

guished, however, from the complete necrosis—caseation—of tuberculosis by the persistence of reticulin and abundance of denatured, structureless "hyalinized" collagen. A search for the recently recognized subgroups of collagen, in particular type I and type III, may prove of value in the further distinction. The complete replacement of the active cellular granuloma by focal hyalinized collagen may be the only clue to old healed sarcoidosis.

A variety of cell inclusions, in particular Schaumann and asteroid bodies, are common in, but not confined to, sarcoidosis.[9] As with focal hyaline scars, the presence of Schaumann bodies is useful in the diagnosis of sarcoidosis even in the absence of cellular granulomas.

A notable feature of sarcoidosis in lymphoid and other tissues is the combination of old scarring, as above, with "fresh" cellular granulomas. This supports the thesis that the unknown cause persists and cannot be degraded by the cellular defense mechanisms, including macrophages; it raises the question whether sarcoidosis is indeed a disease of defective, impotent macrophages.

These cellular details of the sarcoid granuloma are also found in noncaseating tuberculosis, chronic beryllium disease, Crohn's disease, and extrinsic allergic alveolitis, but the pattern and distribution of granulomas within lymph nodes is frequently different in the various diseases. In sarcoidosis the granulomas are widely distributed throughout the node and primarily start in the interfollicular T cell zones. Coalescence and fusion of the granulomas is uncommon.

Although histologic distinction of the granulomas of sarcoidosis from other diseases is not al-

TABLE 8–2 Disorders Associated with Granulomatous Lymph Nodes

- Infections
  - Mycobacteria
    - *M. tuberculosis*
    - *M. kansasii*
    - *M. xenopei*
    - *M. avium*
    - *M. intracellulare*
    - *M. scrofulaceum*
    - *M. leprae*
  - Bacteria
    - *Brucella*
    - *Francisella tularense*
    - *Pseudomonas mallei*
    - *Proprioni*
    - *Yersinia*
  - Spirochaetes
    - *Treponema pallidum*
  - Fungi
    - *Blastomyces*
    - *Coccidioides immitis*
    - *Histoplasma capsulatum*
    - *Cryptococcus neoformans*
  - Protozoa
    - *Leishmania*
    - *Toxoplasma*
  - Other
    - Cat scratch disease
    - *Schistosoma*
    - *Filaria*
- Chemicals: beryllium
- Immunologic disturbance
  - Sarcoidosis
  - Crohn's disease
  - Primary biliary cirrhosis
  - Hypogammaglobulinemia
  - Whipple's disease
  - Immune complexes
- Enzyme defect: chronic granulomatous disease of childhood
- Neoplasia
  - Reticulosis
  - Carcinoma

ways possible, let us compare them with other granulomatous lymph node disorders (Tables 8–2, 8–3, and 8–4).

Infections

The granulomas caused by *Mycobacterium tuberculosis* frequently involve only part of the lymph node, usually coalesce, and frequently surround a large central area of caseation (Figure 8–2). In patients with a high degree of immunity against tuberculosis the granulomas remain discrete and noncaseating and may be indistinguishable from sarcoidosis. Reliance must then be placed on detecting mycobacteria by special staining and by culture. All tissue labeled sarcoidosis must be cultured for mycobacteria, and care must be taken to adjust culture temperatures to meet the requirements of various mycobacteria.

Enlargement of lymph nodes is a feature of primary tuberculosis infecting hilar lymph nodes or *M. bovis* infecting cervical and mesenteric lymph nodes. Secondary or reinfected tuberculosis does not cause enlargement of lymph nodes. The spleen is involved when there is miliary spread from both primary and secondary infection.

M. balnei, causing "swimming pool" granulomas, infects axillary and inguinal lymph nodes. The draining lymph nodes are heavily involved with extensive caseation, whereas the primary skin infection may be inconspicuous. The microscopic picture is similar with *M. kansasii, M. xenopei, M. avium,* and *M. scrofulaceum,* but plasma cells are more evident.

Lymphadenopathy is a feature of lepromatous leprosy (caused by the agent *M. leprae*). There are prominent, large multinucleate lepra cells containing easily identified bacilli. Epithelioid cell granulomas are not a feature of lepromatous leprosy, but rather of tuberculoid leprosy, in which lymphadenopathy is less of a feature.

Brucella causes loose collections of macrophages admixed with polymorphonuclear leukocytes, plasma cells, and occasional eosinophils. The granulomas are not compact, as in sarcoidosis, and Langhan-type giant cells and caseation are both inconspicuous or exceptional.

Yersinia organisms cause granulomatous mesenteric lymphadenitis. Numerous epithelioid cells are arranged around many areas of necrosis containing eosinophils and numerous polymorphonuclear leukocytes to give the picture of a pseudoabscess. Discrete focal granulomas are unusual. The same features are seen in the lymph nodes in cat scratch disease, which may be causally related.

Francisella tularense is acquired from rabbits, hares, and squirrels, possibly via tick bites. The histology is similar to yersinia infection with palisading epithelioid cells surrounding a pseudoabscess.

Pseudomonas mallei causes glanders in horses, but rarely affects man. The histology is similar

TABLE 8–3 Cellular and Other Features of Lymph Node Granuloma

Disease	Focal Granulomas	Necrosis	Polymorphonuclear Leukocytes	Plasma Cells	Eosinophils	Schaumann Bodies Present (%)	Other Features
Sarcoidosis	+++	− to ±	−	−	−	80	Calcification rare
Tuberculosis	+++ (confluent)	+ to +++ caseation	−	−	−	<2	Calcification frequent
Tuberculoid leprosy	±	−	−	−	−	−	—
Hodgkin's disease	+ to +++	−	+	+	++	−	Tumor cells
Chronic beryllium disease	+++	− to ++	−	−	−	60	—
Histoplasmosis or cryptococcosis	++	+ to +++	+ to +++	+	+	−	Calcification frequent
Toxoplasmosis or leishmaniasis	+ to ++	±	±	+	+	−	Small isolated foci of histiocytes
Infectious mononucleosis (EBV)	+	−	−	++	−	−	—
Syphilis	±	− to +++	−	+++	−	−	Vasculitis
Bacterial	+	− to +++	+ to +++	+	±	−	Yersinia or propioni abscess

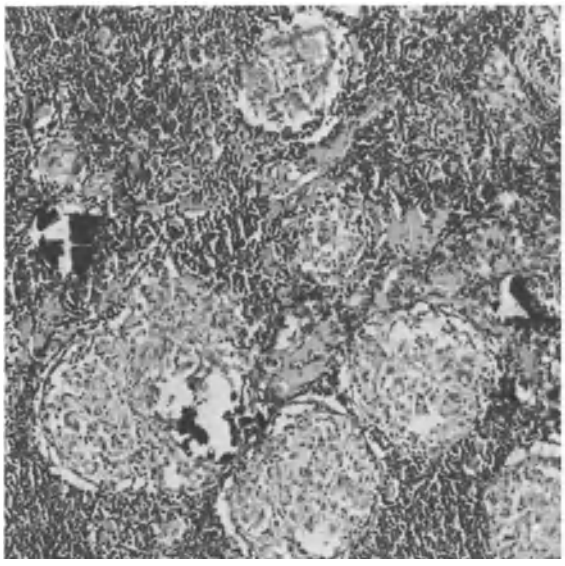

FIGURE 8–1 Sarcoid lymph node showing numerous noncaseating discrete granulomas with three Schaumann bodies. Hematoxylin and eosin stain, ×51.

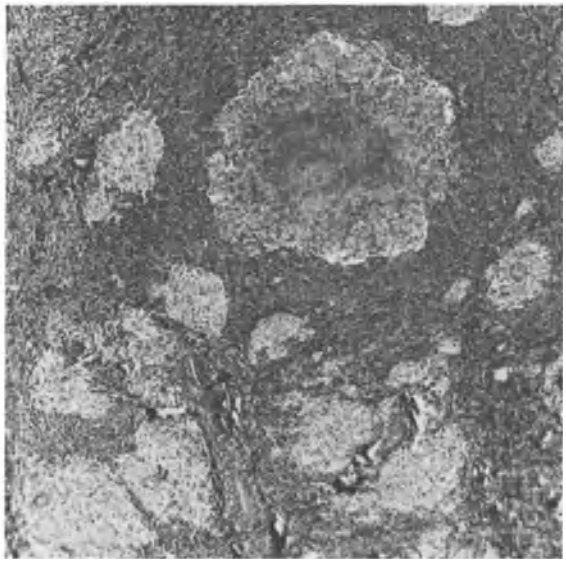

FIGURE 8–2 Tuberculous lymph node showing caseating central granuloma. Note that surrounding discrete granulomas could be mistaken for sarcoidosis. Hematoxylin and eosin stain, ×26.

TABLE 8–4 Differences Between Sarcoidosis and Tuberculosis

Features	Sarcoidosis	Tuberculosis
Age (years)	20–50	Any
Fever	Rare	Common
Weight loss	Rare	Common
Erythema nodosum	Common	Uncommon
Uveitis, skin involvement, enlarged parotids, enlarged lacrimals, bone cysts	Common	Very rare
Involvement of pleura, pericardium, peritoneum, small intestine, meninges	Very rare	Common
Ulceration and sinuses	No	Common
Caseation	Minimal	Maximal
Tuberculin test	Negative in 66%	Positive in most
Lymphocyte blastic transformation	Depressed	Augmented or normal
Kveim-Siltzbach test	Positive in 78%	Negative
Angiotensin-converting test	Elevated	Normal
Hypercalcemia	Yes	No
Hypercalciuria	Yes	No
Hilar lymphadenopathy	Bilateral	Unilateral
Ghon focus	No	Yes
Pulmonary cavitation	Rare, late	Common, early
Calcification	Rare	Common
Corticosteroids	Helpful	Harmful alone
Antituberculous drugs	Unhelpful	Curative
Bacillus Calmette-Guerin vaccination protective	No	Yes

to that caused by *Yersinia,* with considerable necrosis surrounded by epithelioid and a few giant cells.

Generalized lymphadenopathy is a feature of secondary syphilis (caused by the agent *Treponema pallidum*). The distinguishing features are the excess of plasma cells, paucity of giant cells, and lack of necrosis. (If the lymph node has been subjected to the Jarisch-Herxheimer reaction, then necrosis is evident and may resemble caseating tuberculosis.)

Toxoplasma is one cause of glandular fever. The lymph node appearance may be indistinguishable from sarcoidosis. Scattered isolated macrophages may give a starry-sky appearance.

Leishmania causes a generalized lymphadenopathy associated with hepatosplenomegaly. Single or focal collections of macrophages are seen to contain Leishman-Donovan bodies. Unless special stains are done, there may be superficial confusion with sarcoidosis or tuberculosis.

Ova of *Schistosoma* may be embedded within an epithelioid cell granuloma. Giant cells are of multinucleate giant cell type and eosinophils are conspicuous. In a late stage, calcified ova may be buried in fibrous nodules; they are distinguished from Schaumann bodies because they lack the aggregated spherules and crystalline material.

Histoplasma capsulatum causes hilar and peripheral lymphadenopathy. Histoplasmosis mimics sarcoidosis clinically, in the chest radiograph, and in lymph node tissue sections. The organism is observed within macrophages and epithelioid cells with the help of PAS and silver staining. Similar superficial confusion may be associated with *Coccidioides immitis* and *Cryptococcus* neoformans. Caseous necrosis is such that at first glance histology favors tuberculosis rather than sarcoidosis.

Chronic Granulomatous Disease of Childhood

Killing of bacteria depends on a burst of respiratory enzyme activity, which leads to the production of hydrogen peroxide and superoxide in phagocytes. Neutrophils in chronic granulomatous disease of childhood are unable to kill some ingested bacteria because they are deficient in enzymes needed for this superoxide respiratory burst. These defective enzymes may be nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase, cytochrome B, pyruvate kinase, glucose-6-phosphate dehydrogenase, each perhaps contributing a different clinical profile. The problem might also be a lack of lysozyme or lactoferrin.

The classical X-linked disorder occurs in boys about 5 years of age, presenting with hepatosplenomegaly, generalized lymphadenopathy, weeping granulomatous skin lesions, and diffuse miliary lung infiltration. The histology is of multisystem sarcoid granulomas. There may be more than one X-linked form and also more than one autosomal recessive variety. The group is heterogeneous because there are now reports of defective oxidative metabolism with phagocytic stimuli such as bacteria, but not with nonphagocytic soluble metabolic stimuli such as sodium fluoride or concanavalin A. There is certainly more than one mechanism for initiating oxidative metabolism.

Patients with chronic granulomatous disease of childhood only suffer from catalase-producing staphylococci and enterobacteria. Organisms that lack catalase supply the neutrophil with the hydrogen peroxide for their own destruction. Thus catalase-negative organisms, such as pneumococci or streptococci, present no problem to these patients.

Neutrophil leukocytes of normal patients with bacterial infections reduce nitroblue tetrazolium from colorless to form blue-black formazan granules in the cytoplasm. This fails to occur in the leukocytes of children with chronic granulomatous disease of childhood or in the mothers of the X-linked variety.

Neoplasia

Many tumors are granuloma forming in the tumor itself or in the regional lymph nodes. Moreover, radiotherapy and cancer chemotherapy may contribute to granuloma formation. Well-recognized granuloma-producing tumors are pinealoma, dysgerminoma, seminoma, malignant nasal granuloma, and reticulum cell sarcoma. In practice, difficulty in interpretation arises when the pathologist is given insufficient biopsy material.

The greatest confusion leading to the most unfortunate results is that between sarcoidosis and Hodgkin's disease (Table 8–5). Intrathoracic Hodgkin's disease most frequently involves the upper mediastinum; the subcarinal and posterior mediastinal nodes are seldom involved without enlargement of the upper mediastinal nodes. The hilar nodes tend to fuse with the right cardiac

TABLE 8–5 Differences Between Sarcoidosis and Hodgkin's Disease

Features	Sarcoidosis	Hodgkin's Disease
Age (years)	20–40	20–40
Sex (M:F)	Equal	Males 3:1
Weight loss	Rare	Common
Splenomegaly	Rare	Frequent
Submental lymph nodes	Infrequent	Frequent
Erythema nodosum	Frequent	Rare
Gut symptoms	Absent	Common
Pruritus	Absent	Common
Bone lesions	"Punched out" phalangeal cysts	Sclerotic lesions of spine and pelvis
Sclerotic lesions	No	Mixed sclerotic/osteolytic in 16%
Leukocytosis, lymphopenia, eosinophilia	Not a feature	May be present
Hilar lymphadenopathy	Bilateral	Bilateral or unilateral
Hilar gland pressure symptoms	No	Yes
Pulmonary infiltration	Common	Uncommon
Pulmonary infiltration followed by hilar adenopathy	Never	Yes
Pleural effusion	Rare	Common
Skin lesions	Distinctive histology	Specific histology with eosinophilia
Secondary infections	Rare	Common
Abdominal lymphangiogram	Normal	Abnormal
Delayed-type hypersensitivity	Depressed	Depressed
Immunoglobulins	Abnormal	Normal
Kveim-Siltzbach test	Positive	Negative
Radiotherapy	Unhelpful	Curative
Corticosteroids	Therapy of choice	Helpful
Immunosuppressive regimens	Not indicated	Indicated
Prognosis	Good	Guarded

border, whereas in sarcoidosis the right hilar nodes stand away from the right border of the heart.

Mineral Particles

Even in the absence of overt exposure, and with no evidence of silicosis, granulomatous reaction to silica is a common finding in mediastinal and other lymph nodes and in inguinal glands following road accidents. The brightly birefringent crystalline particles are readily identified with polarized light (which is essential for all examinations of granuloma-containing material). The highly fibrogenic silica is surrounded by whorled fibrotic nodules.

Talc is a persisting indigestible material that stimulates macrophage production, resulting in focal collections of cells, including foreign-body giant cells. Talc is recognized by its birefringence and the platelike shape of the crystals.

Chronic beryllium disease closely mimics sarcoidosis, but enlargement of hilar lymph nodes is rare. The epithelioid cell granulomas are histologically indistinguishable from those of sarcoidosis and can affect any organ. Diagnosis is determined by a history of exposure, the finding of beryllium in the tissues, and a positive *in vitro* lymphocyte transformation test.

Immunologic Disorders and Lymph Node Granulomas

Immunoblastic (angioimmunoblastic) lymphadenopathy is characterized by replacement of the architecture by immunoblasts (primitive plasma, B lymphocyte cells), with proliferation of small

blood vessels, frequently showing small epithelioid cell foci. The presence of multinucleate plasma cells, eosinophils, and polymorphonuclear leukocytes, may cause confusion with Hodgkin's disease. The disease is associated with polyclonal hypergammaglobulinemia. It has a poor prognosis.

Hypogammaglobulinemia is a rare immune deficiency state that may affect both B and T cell areas of lymph nodes with a loss of follicles and paracortical cells, respectively. Scanty, small foci of epithelioid cells may occasionally be seen but could well be due to infection by unidentified organisms, in particular Toxoplasma, to which these patients are particularly prone.

Alimentary Tract Lymph Node Granulomas

Lymph nodes in Crohn's disease show isolated granulomas in about 20% of cases. Necrosis is uncommon, Schaumann bodies are rare, and there may be extensive edema and inflammatory hyperplasia.

Primary biliary cirrhosis may have granulomas in portal tract lymph nodes. They occur as isolated, small granulomas, unassociated with necrosis. There may be superficial diagnostic confusion when granulomas are found in abdominal lymph nodes, liver, and lung.

Other unusual causes of granulomas in mesenteric lymph nodes include cholegranulomatous lymphadenitis, cystic pneumatosis, and Whipple's disease. Cholegranulomatous lymphadenitis is distinguished by the presence of bile and macoproteins within small, diffuse foci of foamy macrophages that only rarely take the form of sarcoid epithelioid cell granulomas. The changes result from leakage of gall bladder contents in longstanding chronic cholecystitis. Similar changes are occasionally found within the liver in relation to obstructed bile ducts and may then cause confusion with granulomas found in primary biliary cirrhosis. Cystic pneumatosis is an ill-understood phenomenon in which gas cysts (unassociated with gas-producing organisms) affect the intestinal wall and draining lymph nodes. Macrophages and epithelioid cells surround the cysts, sometimes with the formation of Langhan-type giant cells with occasional eosinophils. Similar changes may occur around lipid-containing cysts in Whipple's disease, which is characterized by prominent periodic acid–Schiff staining histiocytes.

Miscellaneous Granulomatous Lymphadenopathy

In dermatopathic lymphadenitis lymph nodes draining chronic skin diseases contain epithelioid and Langhan-type giant cell granulomas mixed with sinus histiocytes and distinguished by the presence of melanin and lipid pigments and prominent eosinophils. The alternative name "lipomelanic reticulosis" emphasizes these pseudolymphomatous features.

Silicone lymphadenopathy is a reaction to silicone particles in lymph nodes draining prostheses. Focal collections of macrophages and giant cells superficially mimic sarcoid granulomas but are distinguished by the presence of faintly birefringent organic silicone.

Lymphangiographic lymphadenopathy is a result of reactive changes to commonly used radioopaque solutions such as Lipiodol. Macrophages, foreign-body giant cells, and eosinophils accumulate around lipid-filled cysts. Similar changes may be caused by oily media used for "depot" injections.

Drug-induced granulomatous lymph nodes are chiefly caused by anticonvulsant drugs, particularly hydantoin, and anti-inflammatory drugs such as phenylbutazone. The changes mimic lymphomas, in particular immunoblastic lymphomas; these occasional isolated giant cells and small macrophage foci seldom if ever mimic the widespread focal granulomas of sarcoidosis.

"Allergic" granulomatous lymphadenitis results when lymph nodes are the site of polyarteritis nodosa, Wegener's granulomatosis, the Churg-Strauss syndrome, or lupus erythematosus. Necrosis is usually conspicuous, vasculitis is constant, and eosinophils are prominent. In some cases epithelioid and Langhan-type giant cells are present and directly related to vessel necrosis and fragmented elastica. The giant cells in Wegener's granulomatosis are small and contain eosinophilic cytoplasm and many fixed necrotic nuclei.

Clinical Features

Sarcoidosis is a multisystem disease. Data from a world-wide survey of 3676 patients attending 11 sarcoidosis clinics in 9 different countries show that involvement of lymph nodes and spleen is

just part of widespread sarcoid involvement of intrathoracic tissues, eyes, skin, parotids, nervous system, and bone (Table 8–6). In the world-wide survey, peripheral lymphadenopathy and splenomegaly occurred in 1031 of 3676 (28%) patients with sarcoidosis, and in the Royal Northern Hospital, London,[6] peripheral lymphadenopathy was noted in 225 (27%) and splenomegaly in 101 (12%) of 818 patients (Table 8–6).[11]

The histology of noncaseating epithelioid cell granulomas may be highly suggestive of sarcoidosis, but it is insufficient for diagnosis on its own. The diagnosis of sarcoidosis is a synthesis of an appropriate multisystem clinical picture, a suggestive chest x-ray abnormality, histologic evidence, and, preferably, other markers of the disease.

It should always be considered when a patient in the 20–40 year old age group is attending pulmonary, dermatology, and ophthalmology clinics, for these are the disciplines in which it is most often seen. General examination of the patient must be thorough. It is incomplete unless accompanied by a chest radiograph, slit-lamp examination of the eyes, and estimation of the 24-hour urine calcium output. Clinical features include eye involvement, skin lesions, neurosarcoidosis, myocardial sarcoidosis, and bone cysts.

Eye involvement includes acute and chronic anterior and posterior uveitis, conjunctival follicles, scleritis, and lacrimal gland enlargement. These are often interwoven into well-defined patterns of disease. Acute iritis, erythema nodosum, and hilar adenopathy have a benign, self-limiting course. Lupus pernio, associated with chronic uveitis, pulmo-

TABLE 8–6 Comparison of Sarcoidosis in London Series with the World-Wide Survey

Sarcoidosis Features	Royal Northern Hospital, London		World-Wide Survey[a]	
	No.	%	No.	%
Total	818	100	3676	100
Female	500	61	2082	57
Presentation under 40 years	604	74	2504	68
White	728	89	—	—
West Indian	81	10	—	—
Intrathoracic	700	88	3224	87
Erythema nodosum	251	34	640	17
Lupus pernio	34	4	—	—
Other skin lesions	113	14	324	9
Ocular lesions	224	27	539	15
Parotid enlargement	52	6	160	4
Nervous system	77	9	134	4
Bone	31	3	109	3
Lymph nodes and spleen	326	39	1031	28
Lymphadenopathy	225	27	—	—
Splenomegaly	101	12	—	—
Hepatomegaly	82	10	—	—
Heart	27	3	—	—
Lacrimal gland	22	3	—	—
Kidney	10	1	—	—
Upper respiratory tract	53	6	—	—
Systemic corticosteroid therapy	344	42	1738	47
Mortality due to				
Sarcoidosis	25	3	84	2.2
Other causes	23	3	54	1.4
Skin tests				
Positive Kveim-Siltzbach	550/657	84	1714/2189	78
Negative tuberculin test	488/702	70	2093/3268	64
Hyperglobulinemia	161/526	31	808/1832	44
Hypercalcemia	99/547	18	200/1760	11

[a] See ref. 6.

nary fibrosis, and bone cysts, is persistent and troublesome. Keratoconjunctivitis sicca, with or without parotid and lacrimal gland enlargement, anterior uveitis, and facial palsy, constitutes Heerfordt's syndrome (uveoparotid fever).

A major skin lesion is erythema nodosum. It is associated with bilateral hilar lymphadenopathy, polyarthralgia, and occasionally acute iritis. It predominates in women of childbearing age and is often associated with pregnancy or lactation, suggesting a hormonal factor in its genesis. Transient circulating immune complexes may be detectable in this phase of sarcoidosis. Lupus pernio is a chronic, persistent, violaceous lesion with a predilection for the nose, cheeks, and ears. It reflects chronic fibrotic sarcoidosis. It develops insidiously and progresses indolently over the years. Other skin lesions include persistent plaques, maculopapular eruptions, scars, and keloids. The keloids may be found at the sites of earpiercing, surgery, repeated venesection in the antecubital fossa, or even tuberculin and other skin tests.

Neurosarcoidosis occurs in about 4%–7% of patients. Facial palsy is the most frequent presentation, either alone or with other cranial nerve palsies or with papilloedema. Other features include peripheral neuropathy, myopathy, meningitis, space-occupying brain lesions, epilepsy, cerebellar ataxia, hypopituitarism, and diabetes insipidus. Neurosarcoidosis has a mortality of 10%, which is twice the overall mortality of sarcoidosis. Younger patients with erythema nodosum are more likely to respond to corticosteroids than those with space-occupying intracranial mass, chronic skin lesions, chronic uveitis, or pulmonary fibrosis.

Myocardial sarcoidosis is difficult to recognize clinically. It should be considered if a patient with florid multisystem sarcoidosis develops bundle-branch block, arrhythmias, congestive cardiac failure, pericarditis, or clinical evidence of cardiomyopathy. Sudden death without previous evidence of a heart lesion may occur, particularly in patients over 40 years of age. Macroscopic changes are most evident in the posterior part of the ventricular septum. Microscopy discloses granulomas, fibrous tissue, or an admixture.

Sarcoidosis of bone most often involves hands or feet, but very occasionally temporal or frontal bones or the hard palate. It is associated with soft tissue swelling, joint stiffness, and pain. There is no correlation between bone involvement and abnormal calcium metabolism. There are three radiologic types of bone lesions: (a) In permeative lesions progressive cortical tunneling is seen with remodeling of trabecular and cortical architecture. (b) In lytic lesions punched-out cysts are associated with pressure narrowing of the shaft, pathologic fractures, and residual cavities. (c) In destructive lesions, rapid destructive change causes secondary joint surface involvement, and this may be followed by a periosteal reaction.[11]

Chest Radiography

It is customary to stage intrathoracic sarcoidosis according to these chest x-ray changes on first presentation: stage 0, clear chest x-ray; stage 1, hilar lymphadenopathy; stage 2, hilar lymphadenopathy and pulmonary infiltration; and stage 3, pulmonary infiltration without hilar adenopathy. This is a crude classification with much overlapping and we are all trying to improve it. Despite its many shortcomings, it is simple and, most importantly, it is recognized and understood throughout the world.

The chest x-ray changes in a homogeneous Royal Northern Hospital series are compared with those observed in a heterogeneous world-wide survey in Table 8–7. In stage 1, bilateral hilar lymphadenopathy is the most frequent presentation, and eventually about three-fifths of the patients achieve a clear chest radiograph. Bilateral hilar lymphadenopathy was noted in 438 patients presenting with a stage 1 chest radiograph in the London series. Hilar adenopathy subsided eventually in 268 of 458 (59%). The course of the remaining 190 was variable. Pulmonary infiltration developed in 92, and this persisted as the hilar glands subsided in 30 instances. Steroid therapy was not related to the outcome, for it was given to two-thirds of those who had persistent x-ray changes and only one-third of those who attained chest x-ray resolution.

Hilar adenopathy was accompanied by pulmonary infiltration (stage 2) on presentation in 150 patients, with eventual resolution in 59 (39%). Persistent changes in the remainder consisted equally of infiltration with or without adenopathy. Steroid therapy again had little influence on the outcome: steroids were used with equal frequency in those who had persistent changes and in those achieving resolution. Pulmonary infiltration without adenopathy (stage 3) was noted on presentation in 92 patients, and 35 (38%) achieved subse-

TABLE 8–7 Intrathoracic Involvement on Chest X-Ray and Eventual Resolution in London Series and in World-Wide Survey

Sarcoidosis Features	Royal Northern Hospital Series[a]		World-Wide Survey	
	No.	%	No.	%
Total	818	100	3654	100
Stage at presentation				
0	118	13	277	8
1	458	65	1865	51
2	150	22	1066	29
3	92	13	446	12
Eventual chest x-ray resolution (by stage)				
1	268	59	1217	65
2	59	39	490	46
3	35	38	90	20
Overall resolution	362	52	1797	49

[a] See ref. 11.

quent resolution. Steroid therapy again did not correlate with resolution.

Immunology

Acceleration in our understanding of immune mechanisms is due to accumulated data on the cell types found in bronchoalveolar fluid, histochemical and electron microscopic advances, and the identification of the many different enzymes and proteins associated with these cell types. Sarcoidosis is characterized by dysfunction of circulating T cells and overactivity of B cells, and by the frequent presence of circulating immune complexes. When the lungs are invaded by the causative antigen, helper T cells become evident in large numbers at sites of antigen–antibody activity, including bronchoalveolar fluid.[3] This is precisely what might be expected of the body defenses. At this time, the peripheral blood is starved of helper T cells, but it retains a conspicuous number of anergic suppressor T cells. This may partially explain the cutaneous and *in vitro* anergy and, in particular, the negative tuberculin skin test reaction.

Helper T cells stimulate B cells so that all classes of serum immunoglobulins are increased, and circulating antibodies to Epstein-Barr virus, herpes virus, rubella, measles, parainfluenza viruses, *Chlamydia,* and even *Propionibacterium* are significantly increased; also noted have been increased antibody production to mismatched blood and occasionally false-positive Wassermann reactions. A radioimmunoassay technique, easily carried out, now shows that increased kappa and lambda chains also reflect B cell overactivity. They are raised in 79% of patients with active sarcoidosis of more than 2 years' duration, 10% of patients with recent active disease, and only 1% of patients with healed inactive sarcoidosis; they are normal in tuberculosis.

The macrophage is switched on by helper T cells into a frenzy of activity. Macrophages clump together into actively secreting epithelioid and giant cells, which coalesce into a sarcoid granuloma. This activity is measured by (a) increased tissue (TACE), serum (SACE), and bronchoalveolar lavage fluid (LACE) levels of angiotensin-converting enzyme, (b) raised lysozyme, collagenase, elastase, lactate dehydrogenase, and glucuronidase in bronchoalveolar fluid, and (c) increased chemiluminescence. There is a good SACE–LACE correlation for intrathoracic sarcoidosis, but this correlation is poor in widely disseminated sarcoidosis because of still greater elevations of LACE. It is possible to measure not only this enzyme activity, but also protein activity in bronchoalveolar fluid. There is an increase of orosomucoid, α_1-antitrypsin, and protein L1.

Immune complexes are present in about 50% of sarcoid patients and are inversely related to T helper cell levels. Clinical hallmarks are erythema nodosum, polyarthralgia, uveitis, meningism, and a greatly elevated sedimentation rate; but some studies have failed to establish a close correlation between immune complexes, clinical findings, and radiographic stages of sarcoidosis.

Attention is now being increasingly focused on recognition of the cell types involved in sarcoidosis and measurement of their activity. The β_2-microglobulin is a low molecular weight protein (11,800 daltons) that constitutes part of the HLA antigens on the cell surface. Activated T cells increase its production, and this increase can be measured in both blood and bronchoalveolar lavage fluid.

B cell activity can be measured by levels of light chains; activated macrophages by SACE, LACE, collagenase, and lysozyme levels; and polymorphonuclear leukocyte activity by lactoferrin levels. Histochemistry may differentiate acute from chronic sarcoidosis in crysostat sections. High activity of acid phosphatase and leucine aminopeptidase indicates rapid resolution of the sarcoid process and a good prognosis. Killer and natural killer cell activity indicates high activity, particularly in advanced pulmonary disease.

The uptake of gallium-67 is a measure of macrophage activity, correlating with T cell activity, and is yet another dimension of sarcoid granuloma formation.

Persons who are HLA-A1-B8 are more likely to express their sarcoidosis in the form of erythema nodosum, acute arthritis, and anterior uveitis.[12] This is of some interest because, in general, patients with anterior uveitis or seronegative polyarthritis are most likely to be HLA-B27; this is not so in sarcoidosis. There is also some evidence that when sarcoidosis develops in persons who are HLA-B13, it is likely to be chronic and persistent. This is another aspect that should be resolved by monoclonal antibody studies and fresh means of tissue typing.

The Kveim-Siltzbach skin test was first developed 4 decades ago. By means of intracutaneous inoculations of a heat-killed suspension of a sarcoid lymph node, Kveim obtained, in 1–4 weeks, lesions histologically consistent with sarcoidosis in 12 of 13 patients with sarcoidosis.[10] Siltzbach and Ehrlich[13] reported on the value of the test performed on 571 occasions in 200 patients, and the first British report[17] described it as a simple, safe, and specific outpatient technique for providing histologic proof of sarcoidosis and indexing the activity and progress of the disease.

Since those early days there has been a generation of experience of the course and prognosis of sarcoidosis. A retrospective comparison of a large, intensively investigated world-wide series indicates that the Kveim test remains reliable and helpful on three continents. It was positive in 1714 of 2189 (78%) patients—a very large international experience with an insignificant level of false reactions.

Biochemistry

The chemical abnormality in sarcoidosis that has received the most attention has been the abnormal calcium metabolism, possibly because it is amenable to a treatment that prevents long-term complications. With the introduction of new techniques, it became fashionable to measure serum immunoglobulins, alkaline phospatase, and, more recently, SACE, serum lysozyme, collagenase, and elastase.

Hypercalcemia occurs transiently in about one-fifth and hypercalciuria in about two-fifths of patients with sarcoidosis, but residual, irreversible nephrocalcinosis is a rare event. Serum phosphate levels were recorded in 343 patients: they were above normal levels in 22 (6%) and below normal in 25 (7%).[16] No association was demonstrated between abnormal phosphate levels and deranged calcium metabolism.

In a world-wide survey of sarcoidosis, serum globulins were abnormal in 808 of 1832 (44%) patients tested.[6] In our laboratory, serum globulin levels were raised above 35 g/l in 161 of 526 (31%) patients. Abnormal electrophoretic patterns carried no obvious diagnostic or prognostic significance, as no correlation was found with the clinical or radiographic patterns of the disease.

SACE is elevated in about 60% of patients with active sarcoidosis.[15] A number of methods have been described to measure SACE. Two of the more commonly used assay procedures utilize the substrate analogue hippurylhistidylleucine. A spectrophotometric method described by Cushman and Cheung measures enzyme activity in terms of the rate of release of hippurate.[1] The method described by Friedland and Silverstein is based on the spectrofluorometric detection of the *o*-phthaldialedehyde adduct of the reaction product histidyl-

leucine.[2] In our experience the latter method is more suitable for routine use, being extremely sensitive, and not being affected by lipemic or hemolyzed serum samples. Our normal range of 34 ± 18 nmole/min/ml$^-$ was based upon the mean ± 2 SD of values obtained in healthy adult patients.

There are, however, two reasons it is not very useful in the diagnosis of sarcoidosis. It is only positive in about 60% of patients and, unfortunately, it is not absolutely specific, for there is a 10% false-positive rate. Its great value lies in monitoring the progress of the disease. Angiotensin-converting enzyme is a product of active metabolism by the epithelioid cell granuloma. When activity of this granuloma is suppressed by corticosteroids, SACE falls toward normal. A rising level heralds a relapse and is an indication for further corticosteroid therapy. It is helpful in monitoring the progress of the disease during pregnancy, when chest radiography is undesirable, and this biochemical marker of activity has allowed a reduction of chest radiography. This is the first biochemical marker of activity of sarcoidosis, and it is a more sensitive index of its fluctuations than serial chest radiography. There is a broad correlation between the SACE level and several other test results (Table 8–8).

Serum lysozyme is elevated in a number of conditions, including tuberculosis. It is often elevated in sarcoidosis, in which its measurement correlates with the clinical activity of the disease. Monocytes and tissue macrophages contain high lysozyme activity, and therefore measurement of serum lysozyme may reflect the volume of granulomatous tissue present. It is a feature of acute active sarcoidosis, and serum levels are normal in chronic fibrotic disease. Elevated lysozyme and angiolensin-converting enzyme activities have been demonstrated in sarcoidosis lymph nodes[14]; there is a positive correlation between the two enzymes.

TABLE 8–8 Tests that Correlate and Do Not Correlate with Serum Angiotensin-Converting Enzyme

Positive Significant Correlation	No Significant Correlation
Serum lysozyme	Serum immunoglobulins
Serum collagenase	Sedimentation rate
β-glucuronidase	Tuberculin skin test
T cell lymphocyte activity	
Killer and natural killer cell activity	
Radioactive gallium uptake	
Kveim-Siltzbach skin test	
Sarcoid hypercalcemia	
Chest x-ray resolution	

Course and Prognosis

There are two different types of sarcoidosis. Acute (active) sarcoidosis has an abrupt onset, acute inflammatory lesions, an exudative histologic picture, raised SACE levels, hydroxyprolinuria, a positive Kveim-Siltzbach test, a high incidence of spontaneous remission within 2 years, a good response to steroids and to other anti-inflammatory drugs, and a good prognosis. Chronic sarcoidosis has an insidious onset, (inactive) relapsing course, a fibrotic clinical and histologic picture, normal SACE levels, negative Kveim-Siltzbach test, and a poor prognosis despite treatment (Table 8–1).

The most predictable course is that of erythema nodosum with bilateral hilar lymphadenopathy. The skin lesions subside within 1 month of onset and the hilar adenopathy regresses within 1 year; recurrences and sequelae are rare.

Skin and pulmonary lesions are similar in course and in the response to treatment. Transient skin lesions are usually associated with hilar adenopathy, whereas chronic skin lesions are more likely to be accompanied by pulmonary fibrosis. Transient skin lesions tend to clear without treatment, whereas the chronic ones fail to do so despite prolonged therapy. All types of pulmonary sarcoidosis may be expected to resolve in 60% of patients, but resolution is less likely in older patients and in those in whom there is accompanying extrathoracic disease, particularly bone and skin lesions. The combination of pulmonary and cutaneous sarcoidosis constitutes the hallmark of chronicity, presumably because longstanding fibrosis has supervened. Clearing of the abnormal chest radiograph occurs in only one-third of patients with bone cysts, in one-fifth of those with skin lesions, and in only one-sixth of patients in whom bone and skin lesions accompany pulmonary sarcoidosis.

Sarcoidosis would remain a relatively benign and unimportant disease but for the development of certain devastating complications: irreversible fibrosis in the lungs and eyes, and nephrocalcino-

sis. In assessing the prognosis, it is important to assess the degree of functional failure of the lungs, heart, eyes, and kidneys. In the practical management of sarcoidosis it is the physician's duty to diagnose the condition sufficiently early so that effective therapy may prevent these unfortunate sequelae.

Etiology

There have been many claims that sarcoidosis is an infection, perhaps due to a *Mycobacterium* or virus, but none has been identified. There are striking clinical, radiologic, and immunologic similarities between sarcoidosis and Hodgkin's disease (Table 8–5), but these may be superficial and not causally related. It would be distressing for both the patient and relations to confuse the two conditions.

Many factors contribute to the form and function of the sarcoid granuloma, including prolonged antigenemia, possibly defective macrophage defenses, and circulating immune complexes. Sex, hormones, race, age, and HLA type may be background factors. Many different cell types also contribute (Figure 8–3). The interplay may lead to an acute or insidious attack of sarcoidosis, just as many factors contribute to migraine or asthma.

Treatment

There are four essential steps in the management of a patient suspected of having sarcoidosis:

1. Seek histologic confirmation of the clinical diagnosis as soon as possible. The patients who are troubled in later years by their disease are almost always those in whom the clinical diagnosis was not supported by histologic confirmation initially, and so treatment was delayed until it was too late to be effective. The complications are often more troublesome than the disease.

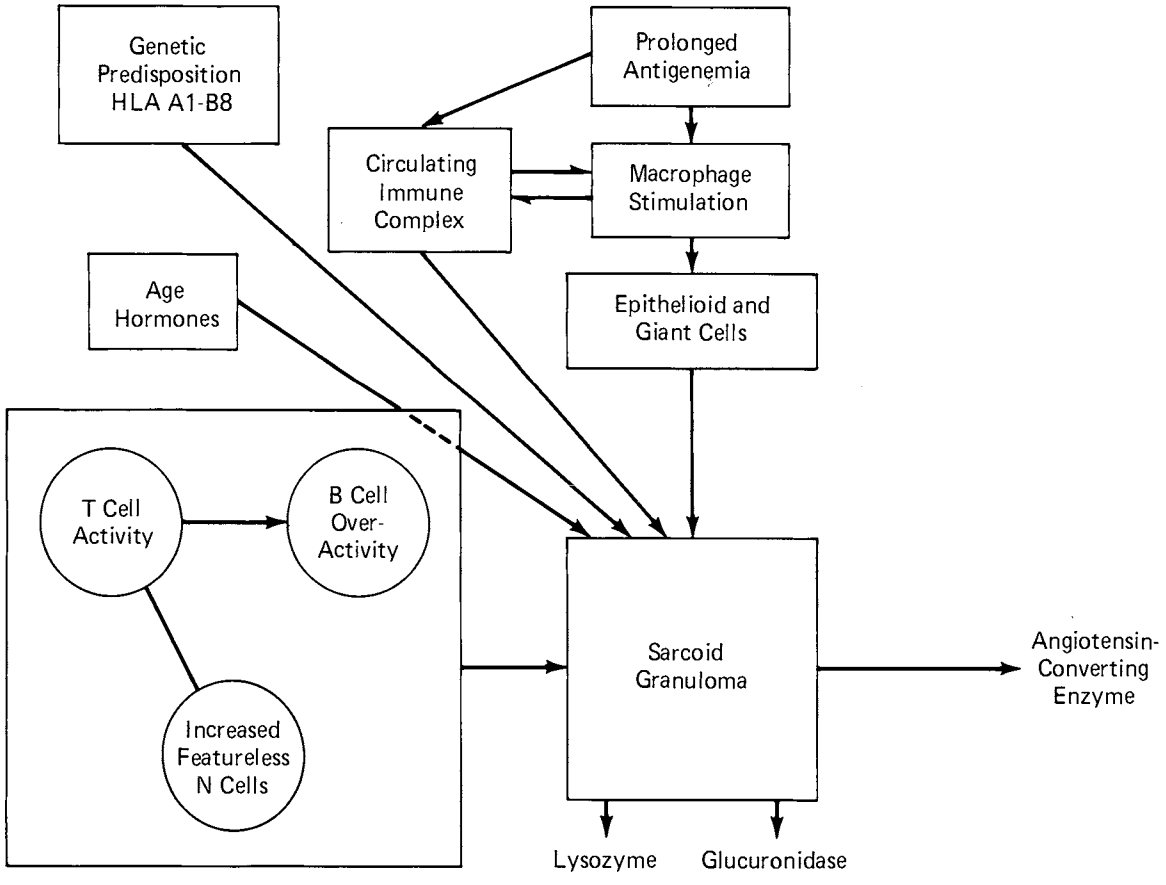

FIGURE 8–3 Factors contributing to the sarcoid granuloma formation.

TABLE 8–9 Differences Between Sarcoidosis (a Multisystem Disorder) and a Nonspecific Local Sarcoid Tissue Reaction

Feature	Multisystem Sarcoidosis	Local Sarcoid Tissue Reaction
Number of systems involved	Several	Usually one
Age (years)	20–50	Any
Chest radiograph	Abnormal in 87%	Normal
Slit lamp examination of eyes	Abnormal in 15%	Normal
Tuberculin skin test	Negative in 66%	Variable
Kveim-Siltzbach skin test	Positive in 78%	Always negative
Serum angiotensin-converting enzyme	Elevated in 60%	Normal
Calcium metabolism	Abnormal in 20%	Normal
Response to corticosteroids	Good	Variable
Other treatments	Indomethacin, oxyphenbutazone, chloroquine, *p*-aminobenzoate, methotrexate	Depends on cause, anti-infective immunosuppressives

2. Make sure that granulomas found in one system are part of the multisystem disorder and not just a nonspecific, local sarcoid tissue reaction. There are several points of differentiation (Table 8–9)—clinical, radiologic and immunologic—but the simplest and most secure way of distinguishing them is by the Kveim-Siltzbach test.
3. Judge whether the disease is acute or chronic (Table 8–1).
4. Decide whether treatment is necessary, and if so, choose the most effective agents and avoid useless, toxic, and obsolete drugs. For instance, although widely recommended for many years, calciferol and antituberculous drugs are useless in the treatment of sarcoidosis. Even more important, it should be remembered that they are dangerous because of their toxicity. There are several therapeutic regimens that should be considered or avoided, but the mainstay continues to be the various forms of corticosteroids.

Indications for Corticosteroid Therapy

Corticosteroid therapy for sarcoidosis is indicated in the following conditions: ocular, neurologic, glandular, myocardial, and upper respiratory tract involvement; breathlessness; persistent hypercalciuria; disfiguring skin lesions; bone cysts; and an abnormal chest radiograph. Treatment for each of these is described below.

Topical corticosteroids should always be administered for iridocyclitis in the form of eye drops applied frequently during the day, reinforced with a corticosteroid eye ointment at night. If there is no substantial and continuing improvement over 10 days, then the concentration of corticosteroid in the anterior segment of the eye may be increased by a local subconjunctival depot of cortisone. Oral corticosteroids are indicated if local treatment does not lead to a rapid response or if ophthalmoscopy reveals posterior uveitis. In addition, the inflamed iris should be rested by local atropine eye drops to maintain a dilated pupil.

An abnormal chest radiograph that does not show any spontaneous improvement in the course of 3–6 months is an indication for corticosteroid therapy. Bilateral hilar lymphadenopathy is likely to subside without treatment, particularly if it is associated with erythema nodosum. On the contrary, pulmonary infiltration that remains static or worsens during the course of 6 months is an indication for oral steroids in an attempt to prevent the development of irreversible pulmonary fibrosis. Several factors influence the likelihood of resolution of the chest radiograph. The younger the patient the more likely it is to clear. The presence of chronic skin lesions and/or bone cysts would confer an unfavorable prognosis, for these are the hallmarks of chronicity. Their presence would suggest that the pulmonary lesions are unlikely to subside. Substantial improvement in the chest ra-

diograph is also related to the length of time before the treatment is given. Results are considerably better in those treated within 2 years of onset of the disease.

If breathlessness is present, then the patient has already reached a stage of irreversible pulmonary fibrosis or disturbed gas transfer. Oral steroids provide symptomatic relief but do not influence the natural history of the disease or its grave prognosis at the irreversible stage of breathlessness. At this stage, of course, the aim of treatment is to improve pulmonary function rather than chest x-ray appearances.

In the presence of persistent hypercalciuria, steroids can be used to prevent excessive gastrointestinal absorption and excessive urinary excretion of calcium. Instead, calcium is diverted to a harmless fecal excretion within days of the exhibition of oral steroids.

When lupus pernio or other unsightly skin lesions distress patients, oral and local corticosteroids may correct the deformity. Unfortunately, small doses of steroids must be continued indefinitely if the cosmetic improvement is to be maintained. In order to minimize the side effects of oral corticosteroids, local intralesional triamcinolone acetonide, chloroquine, or both are helpful alternatives.

In the case of neurologic involvement, the more acute the disease, the more likely it is to respond to systemic corticosteroids, which should be administered as soon as the diagnosis has been established. Resolution of neurosarcoidosis is more likely to occur in younger patients whose sarcoidosis has an explosive, sudden onset. The response to treatment is better in those with accompanying erythema nodosum rather than chronic skin lesions, those with acute rather than chronic uveitis, and those with hilar adenopathy rather than old, diffuse, pulmonary infiltration.

Corticosteroids are useful in the treatment of glandular involvement, particularly if there is disordered function, e.g., dry eyes due to lacrimal gland involvement, dry mouth due to salivary gland enlargement, and hypersplenism due to sarcoidosis of the spleen. The indication for treatment is disordered function rather than anatomic enlargement of the gland in question.

It is easy to include involvement of the heart in a theoretical list of indications for corticosteroid therapy, but much more difficult in practice to recognize myocardial sarcoidosis. It should be suspected if a patient with florid, multisystem sarcoidosis develops heart block, bundle-branch block, arrhythmias, congestive cardiac failure, pericarditis, or clinical evidence of cardiomyopathy. Sudden death without previous evidence of a heart lesion may occur. Sarcoid granulomas of the myocardium seem to occur most frequently in females over 40 years of age. Macroscopic changes are most evident in the posterior part of the ventricular septum. Microscopy discloses granulomas, fibrous tissue, or a mixture of both. Treatment demands steroids for resolution of the granulomas, chloroquine as an antifibrotic agent, beta-blocker antiarrhythmic drugs, and quinidine.

Bone cysts cause pain, swelling, and deformity of hands and feet. They are chronic, persistent manifestations of sarcoidosis occurring with pulmonary fibrosis, lupus pernio, and chronic uveitis. Steroids provide relief of symptoms but bone cysts persist despite treatment, and symptoms unfortunately recur when steroids are discontinued.

Sarcoidosis of the upper respiratory tract involves the nose, nasopharyngeal mucosa, and larynx, causing difficulty or even obstruction with breathing. This obstruction is relieved by steroids. Submucous resection of the nose should not be allowed since the results may be disastrous, with complicating nasal septal perforation and progressive destruction of bone and cartilaginous septum.

Alternative Drugs

Treatment may be necessary when there are contraindications to corticosteroid therapy, or it has proved fruitless. Under these circumstances, it is worth considering treatment with oxyphenbutazone (Tandearil), chloroquine, or potassium *p*-aminobezoate (Potaba).

In a blind controlled trial comparing oxyphenbutazone, prednisolone, and a placebo in the management of pulmonary sarcoidosis, both active drugs were significantly better than the placebo.[5] Prednisolone and oxyphenbutazone were equally effective. Whereas one in six patients showed spontaneous regression of pulmonary sarcoidosis in 6 months, oxyphenbutazone or prednisolone increased the number to one in two patients. The phenylbutazone drugs are anti-inflammatory, so they are indicated for acute exudative sarcoidosis.

They are not helpful in the chronic fibrotic form of the disease.

The way in which the chloroquines act is unknown, but they control some instances of chronic fibrotic sarcoidosis involving lungs and skin. Such treatment is particularly helpful in the management of lupus pernio and pulmonary fibrosis, in a dose of 200 mg on alternate days for 9 months. It may lead to irreversible retinopathy with blindness, or to reversible keratitis, particularly if used in large doses for over 1 year; its ocular toxicity should always be kept in mind. The 9-month courses should be followed by 6-month drug-free intervals to permit maximum excretion.

Potassium *p*-aminobenzoate (Potaba) is known to have an antifibrotic effect in Peyronie's disease, scleroderma, and rheumatoid arthritis. It is worth considering in pulmonary fibrosis and lupus pernio due to sarcoidosis. The 3-g envules should be taken by mouth four times daily for several months. This form of treatment is an alternative to corticosteroids and chloroquine, and giving all three in rotation helps to overcome the undesirable long-term complications of any one drug.

Azathioprine (Imuran) was administered to ten of our patients and led to clinical improvement in four, chest clearing in two, and improved respiratory function in one. These ten patients had already failed to respond to corticosteroids, oxyphenbutazone, and chloroquine, and thus they can be regarded as having hard-core, chronic fibrotic sarcoidosis that resisted all other therapy. Israel had the same indecisive response to the use of chlorambucil and methotrexate.[4] To date, there have been no trials of the effect of another immunosuppressive, antilymphocytic globulin in sarcoidosis.

Allopurinol is an effective uricosuric agent that has recently been advocated in the management of sarcoidosis. It is certainly helpful when there is renal failure, hyperuricemia, and bone and joint pains due to secondary gout. Under these circumstances it should be considered, in combination with indomethacin, for relief of acute symptoms, and continued alone to neutralize uric acid retention.

Methotrexate is given in a dose of 5 mg once weekly for 3 months. It is most effective in treating chronic skin lesions due to sarcoidosis. It is given once weekly to avoid hepatotoxicity.

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CHAPTER 9

Biochemical and Immunologic Disorders in Diseases of the Lymphoid Tissue

Edward H. Cooper

The emphasis of research into biochemical and immunologic disorders in lymphoproliferative disease has undergone considerable change during the past 20 years. The first phase was to look for biochemical indicators of disease activity, and involvement of the liver, especially in Hodgkin's disease (HD), and to a lesser extent in non-Hodgkin's lymphomas (NHL). As radiologic techniques for the assessment of HD have steadily improved and the advantages of close clinical monitoring become self-evident, the first aim has had less practical importance. On the other hand, in multiple myeloma the increasing sophistication of immunochemistry has greatly increased our knowledge of the biology of the disease so that serum and urine protein analyses provide an essential aid to its assessment.

The second phase, which is still being developed, is to relate laboratory-based information to the immune status of the patient, in particular the behavior of the T lymphocyte and macrophage systems, which are essential for cell-mediated immunity. Except for the simplest of such investigations, this type of assessment is not widely used in clinical practice. Finally, our knowledge is increasing in regard to a number of minor serum protein constituents, such as ferritin[68,69] β_2-microglobulin (β_2-M),[33] and IgE,[4,6] which can be at an abnormal concentration in lymphoid neoplasia. The functional significance of some of them remains an enigma, whereas others (for example β_2-M) have an important role as prognositic indicators, but only in the context of multiple myelomatosis[12,100] and chronic lymphocytic leukemia.[124,125] The main plasma proteins to alter in lymphoid disease are listed in Table 9–1.

In parallel with the developments in immunochemistry and cellular immunology, there has been an explosion of interest in the classification of diseases of the lymphoid system according to the distribution of the subsets of the cell types in the tumors and peripheral blood. The identification of specific protein receptors on the cell surfaces, and the production of appropriate antisera to these key proteins, have brought about a new understanding of the wide spectrum of lymphoproliferative disease and a new dimension in the classification of normal and abnormal members of the lymphoid cell series (Table 9–2). This topic is outside the scope of the present chapter; it is discussed in detail in recent reviews.[21,58,88,121,136]

As with all forms of malignancy, the severity of the biochemical disturbances increases with the tumor burden, and later becomes more complex due to the advent of severe infection, mechanical obstruction of the viscera, and, finally, organ failure. In contrast with many common metastatic cancers, in which the progress is often relentless when they are metastatic, the evolution of disseminated malignant tumors of the lymphoid tissue can be slow and often interspersed with long periods of semistability. These "stable" periods are also reflected in the results of longitudinal measurements of many biochemical variables in the blood.

Acute Phase Reactant Proteins

The acute phase reactant proteins (APRPs) are mainly glycoproteins that alter their plasma concentration in response to stimuli produced by

TABLE 9–1 Main Plasma Proteins That Can Alter in Lymphoid Disease

Protein	Molecular Weight (daltons)	Normal Concentration Range (g/l)
Markers of nutritional status		
Albumin	66,000	35–55
Transferrin	77,000	2–4
Retinol-binding protein	21,000	0.3–0.6
Prealbumin	50,000–60,000	0.15–0.40
Acute phase reactants		
C-Reactive protein	160,000	0.5–5.0 × 10^3
α_1-Acid glycoprotein	44,000	0.5–1.4
Ceruloplasmin	124,000–134,000	0.15–0.60
α_1-Antitrypsin	54,000	2–4
α_1-Antichymotrypsin	69,000	0.3–0.6
Main component of complement		
C3	180,000	0.5–1.2
Fibrinogen	340,000	2.0–4.5
Immunoglobulins		
IgA	150,000	2.0–4.0
IgG	150,000	8–17
IgM	900,000	0.6–2.5
Light chains	22,000 (45,000)	—
IgE	190,000	1–100 × 10^6
Others		
β_2-Microglobulin	11,800	1–3 × 10^3
Ferritin	440,000–600,000	1–580 × 10^6

many forms of tissue injury, acute and chronic inflammation, and cancer. Clearly, as they respond to such a wide variety of stimuli, the response is nonspecific, and within a chronic illness the reaction can be induced by more than one cause.[36,48,75,101]

The APRPs are, in the main, synthesized in the liver parenchymal cells, although it has been suggested that acid α_1-glycoprotein (AGP) can also originate from extrahepatic sources.[53] The main functions of the APRPs and their half-lives are given in Table 9–3. It can be seen that some belong to transport systems and some are serine antiproteases, whereas for others, e.g., C-reactive protein (CRP) and AGP, the functions in vivo remain uncertain. There is often a high correlation between the rise and fall of the levels of various APRPs in disease, but they can become dissociated, especially when there is underlying chronic liver disease or drugs such as estrogens are administered.[34]

The temporal patterns of the APRPs differ: for example, the serum levels of CRP and α_1-antichymotrypsin rise rapidly after infection or wounding, whereas AGP, haptoglobulin, α_1-antitrypsin and ceruloplasmin levels rise more slowly and take longer to return to normal. The disturbances in response to the "inflammatory" stimuli in cancer are more complex and need not involve the whole cascade of APRPs.[36] The main factors influencing the serum concentration of an APRP are shown in Figure 9–1.

CRP is the best known of the APRPs. It was discovered 50 years ago as a serum constituent that reacts with polysaccharides of the cell wall of the pneumococcus. CRP is emerging as one of the key proteins in the immediate defense against the noxious effects of tissue injury, what-

TABLE 9–2 Markers of Human Lymphoid Cell Subpopulations

Lineage	Markers
B cell	
Stem cell	? Alloantigen
Pre–B cells	Intracytoplasmic IgM
	? MRBC receptors
	"Ia-like" antigen (subpopulation)
B cells	Surface immunoglobulin
	MRBC receptors
	Epstein-Barr virus receptor
	IgG Fc receptors
	IgM Fc receptors
	IgA Fc receptors
	IgE Fc receptors
	Complement component receptors
	B cell alloantigens ("Ia-like")
T cell	
Stem cell	? Alloantigen
Pre–T cells	Terminal deoxynucleotidyl transferase
	HTLA
	Peanut agglutinin
T cells	HTLA
	Sheep erythrocyte receptors
	Acid α-naphthyl acetate esterase
	IgG Fc receptors (Tγ)
	IgM Fc receptors (Tμ)
	IgA Fc receptors (Tα)
	IgE Fc receptors (Tε)
	TH2 antigen
	Histamine receptors
	Other alloantigens
Third population, "unclassified" lymphoid cells	IgG Fc receptors (high affinity)
	Complement component receptors (subpopulation)
	Epstein-Barr virus receptors (subpopulation)
	"Ia-like" antigen (subpopulation)

From Gupta and Good,[58] with permission.

ever the cause.[106] Despite its nonspecific nature, there is a revival of interest in this serum protein as a useful clinical indicator of disease activity. The protein is synthesized in the liver[66] and is released into the blood as the result of injury; the normal levels are very low, but can increase several hundred-fold within 24–48 hours after wounding or during an acute infection.

It is now well established that in many forms of tissue damage (such as myocardial infarction[77]),

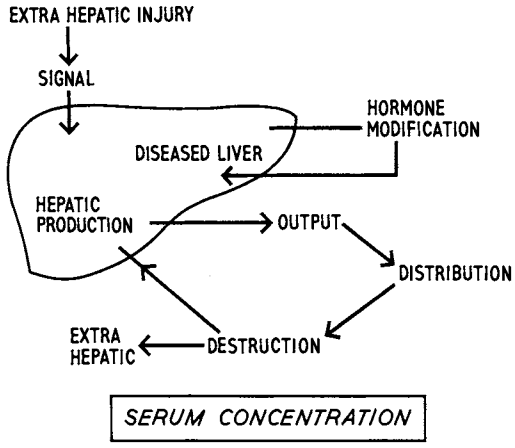

FIGURE 9–1 Main influences that contribute to the serum concentration of an individual APRP.

in uncomplicated wounding, or in successful antibiotic treatment of an infection, there is a rapid, exponential fall in the level of CRP; this function can be helpful when monitoring septicemic complications in hematologic malignancies.[91,113] A wide variety of rheumatoid disorders and cancer may produce a sustained elevation of CRP, which cannot be explained by coincidental infection.[36,78,106] The CRP response is not affected by anti-inflammatory drugs or steroids unless they directly affect the underlying disease.

It is evident that as the site of synthesis of CRP and the other APRPs is usually distant from the site of the inflammatory process, the two sites must be linked by an APRP-inducing mediator. Current knowledge indicates that the build-up of serum CRP levels is associated with a progressive recruitment of liver parenchymal cells synthesizing the protein, proportional to the severity of the injury. There is still uncertainty as to the mediators of the response; several products of the inflammation have been suggested as possible agents, including endogeneous pyrogen and prostaglandin E.

CRP has several properties associated with its ability to bind to phospholipids, sphingomyelin, and phosphatidylcholine,[71] which are constituents of bacterial and mammalian cell walls and their products. In the presence of calcium ions CRP also binds to polycations.[41] In vivo the binding of CRP to a solid phase results in the activation of complement via the classical pathway. The activation of complement then plays a key role in cell lysis and the enhanced phagocytosis, neutrophil chemotaxis, and vascular permeability that are the essential features of an inflammatory response.

TABLE 9–3 Main Acute Phase Reactant Proteins

Protein	Half-life (days)	Main Actions
C-Reactive protein	0.5	Activation of complement; modification of platelet and lymphocyte
Acid α_1-glycoprotein	5.2	Modification of lymphocyte function?
α_1-Antitrypsin	2.5–5.0	Serine antiprotease
α_1-Antichymotrypsin	—	Serine antiprotease
Haptoglobin	2.0–4.0	Formation of complexes with hemoglobin
Ceruloplasmin	5.0–7.0	Binding Cu^{++}, scavenging free radicals
Fibrinogen	2.0–4.0	Formation of fibrin

Acute Phase Proteins in Lymphoproliferative Disease

Ceruloplasmin and Serum Copper

Each molecule of ceruloplasmin contains 6 copper atoms and about 50 carbohydrate residues. The protein has significant oxidase activity as a metalloglycoenzyme; it assists in the mobilization of iron from iron-storage cells in the liver to the plasma and serves as a copper donor to cytochrome oxidase.[51] Ceruloplasmin and serum copper levels may be raised in HD, but there has been much controversy about their interpretation in the past; some investigators thought they might have a place in monitoring the disease.[50,65,97] On the other hand, in children with HD, neither the erythrocyte sedimentation rate (ESR) nor serum copper levels were considered to be a useful guide to disease activity.[137]

Larger retrospective analyses have shown good statistical relationships between serum copper levels and activity in HD.[130] Furthermore, the experience of Kaplan's group in Stanford[138] suggested that serum ceruloplasmin levels are the most promising of various potential markers for monitoring HD. In their series of 100 consecutive untreated HD patients, 78% had a raised serum copper oxidase (ceruloplasmin). Serial measurements over 2 years suggested that this test could be useful in identifying a later recurrence. In all accounts of trace metal determination, the risks of specimen contamination need to be borne in mind, although this is far less of a problem when ceruloplasmin is measured immunochemically. These reports of ceruloplasmin measurement are similar to the experience of repeated measurements of the ESR as a monitor of recurrence of HD reported by Le Bourgeois and Tubiana[82] in Paris, especially when a reasonably high ESR (>30 mm in the first hour) was used as the discriminant value. In a Norwegian study the combination of elevated serum copper and ESR was found in 5% of 78 patients with HD in complete remission and in 61% during an active phase of the disease.[10]

There are fewer reports of copper and ceruloplasmin levels in NHL. Foster et al., using electron spin resonance to assay copper, found only small differences in the serum levels between active and inactive disease with a considerable scatter.[49] However, the same method, when applied to the study of chronic lymphocytic leukemia, indicated the ceruloplasmin levels were related to the tumor mass.[56]

There is a reciprocal relationship between plasma copper and zinc levels, and low levels of zinc have been observed in HD;[26] moreover, the zinc to copper ratio has been found to be well correlated with disease activity in lymphoma.[25]

C-Reactive Protein

The clinical usefulness of CRP measurements in oncology has been reinforced by many investigators. In HD, CRP levels tended to discriminate

symptomatic from asymptomatic forms.[107] In African cases of Burkitt's lymphoma, CRP levels were usually raised in active disease, whereas CRP could not be detected or was very low in children in remission.[92] We have had a similar experience in children with Burkitt's lymphoma in Kenya (E. H. Cooper and Gunvén P., unpublished data). This result is of interest as the various intercurrent parasitic diseases, mainly chronic malaria and various nematodes, did not affect the levels of CRP and the assay is simple and cheap.

There can be discordant results when the ESR and CRP levels are compared; in our series[30] the correlation between these variables was $r = 0.48$; the dissociation of CRP and ESR can also occur in other types of chronic inflammatory disease, such as rheumatic disease.[7] In lymphomas the CRP level tends to be well correlated with other APRPs and the ESR well correlated with the level of plasma fibrinogen.

In contemporary investigations of lymphoma in Western hospitals, the measurement of an APRP phase protein is probably not an important, but it can be very helpful in third world countries. On the other hand, in multiple myeloma, where the risk of infection is very high and onset can be insidious, and the ESR has little meaning, a measurement of CRP can be valuable; it can give warning of a possible episode of infection, which at its onset may be overlooked in the elderly.

The incidence of undoubtedly elevated CRP levels at the time of first presentation and during complete remission in HD and NHL is shown in Table 9–4. Steroids and chemotherapy do not have a direct action on the level of CRP; a fall following treatment indicates the inflammatory processes, whatever their cause, are no longer producing signals that result in an increased rate of CRP synthesis. Examples of the changes in APRPs in lymphomas are shown in Figures 9–2 and 9–3.

Serum Amyloid A Protein

Serum amyloid A protein (SAA) is an APRP that was recently discovered. It was found that secondary amyloid deposits are composed mainly of a low molecular weight protein, which has been named amyloid A (AA).[83] The AA protein consists of a single chain of 76 amino acids with a molecular weight of about 8400 daltons. The SAA protein has complete immunologic identity with the AA protein found in the amyloid deposits. Initial investigation has shown that the SAA protein behaves as an APRP with marked increases of its level (200 to 400-fold) in a variety of inflammatory conditions and pregnancy.[115] SAA levels are increased in secondary amyloidosis as well as in primary and myeloma-associated amyloid disease,[16] and high levels of SAA have been reported in multiple myeloma.[116] SAA is closely bound to the high-density lipoprotein (HDL) fraction of the serum, mainly HDL2 and HDL3; it can be measured by either radioimmunoassay or by modified radial immunodiffusion using a modified Mancini technique.

Current evidence suggests that SAA is synthesized by fibroblasts, plasma cells, neutrophils, and the liver.[77] The only biologic function that has been described for SAA is the inhibition of the in vitro antibody response to T-dependent

TABLE 9–4 Incidence of Raised C-Reactive Protein Levels (>20 mg/l)[a] in Lymphoma

Status and Stage	Hodgkin's Disease		Non-Hodgkin's Lymphoma	
	No./Total	%	No./Total	%
Active disease				
I–II	14/49	28.5	5/22	22.7
III–IV	16/30	53.0	19/48	39.6
Complete remission				
I–II	5/104	4.8	3/41	7.3
III–IV	3/66	4.8	1/58	1.7

[a] The level of 20 mg/l is chosen as an unequivocal rise in C-reactive protein; the normal upper limit is less than 10 mg/l; the distribution of levels in normal subjects is skewed with more than 50% have a level less than 1 mg/l.

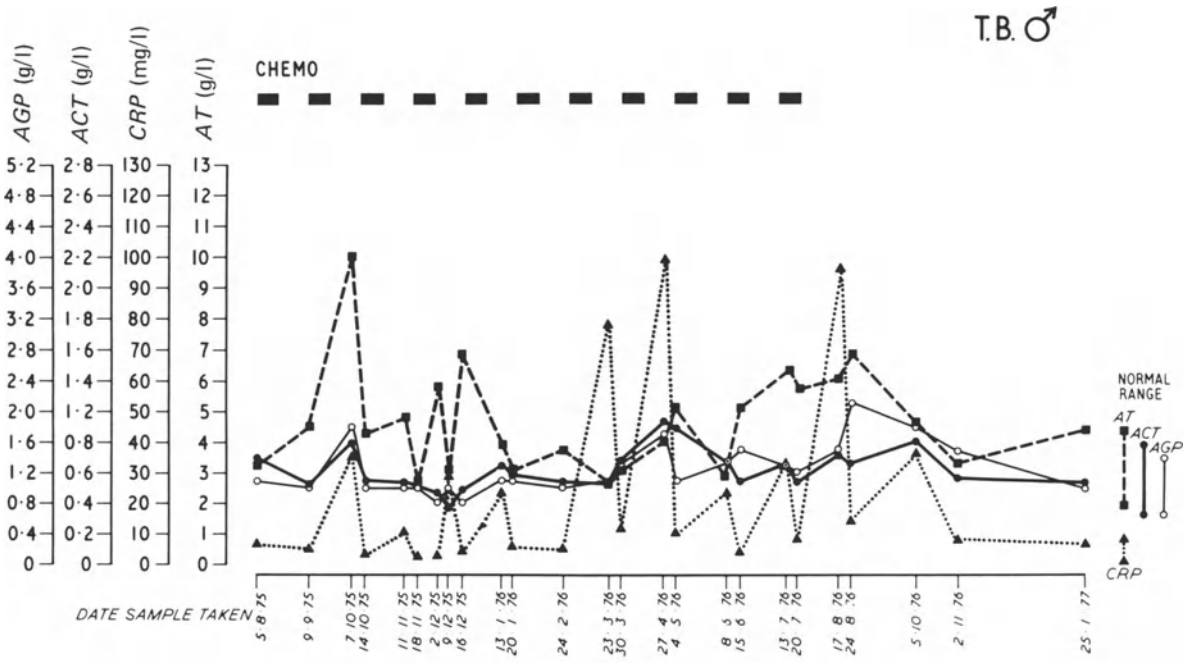

FIGURE 9–2 Changes in APRPs in a 31-year-old man with Hodgkin's disease, diagnosed July 1974; relapse with nodal and systemic symptoms, July 1975. Chemotherapy after relapse resulted in partial remission only. Patient developed leukoencephalopathy and died. AGP, acid α_1-glycoprotein; ACT, antichymotrypsin; CRP, C-reactive protein; AT, antitrypsin. (From Child et al.,[29] with permission.)

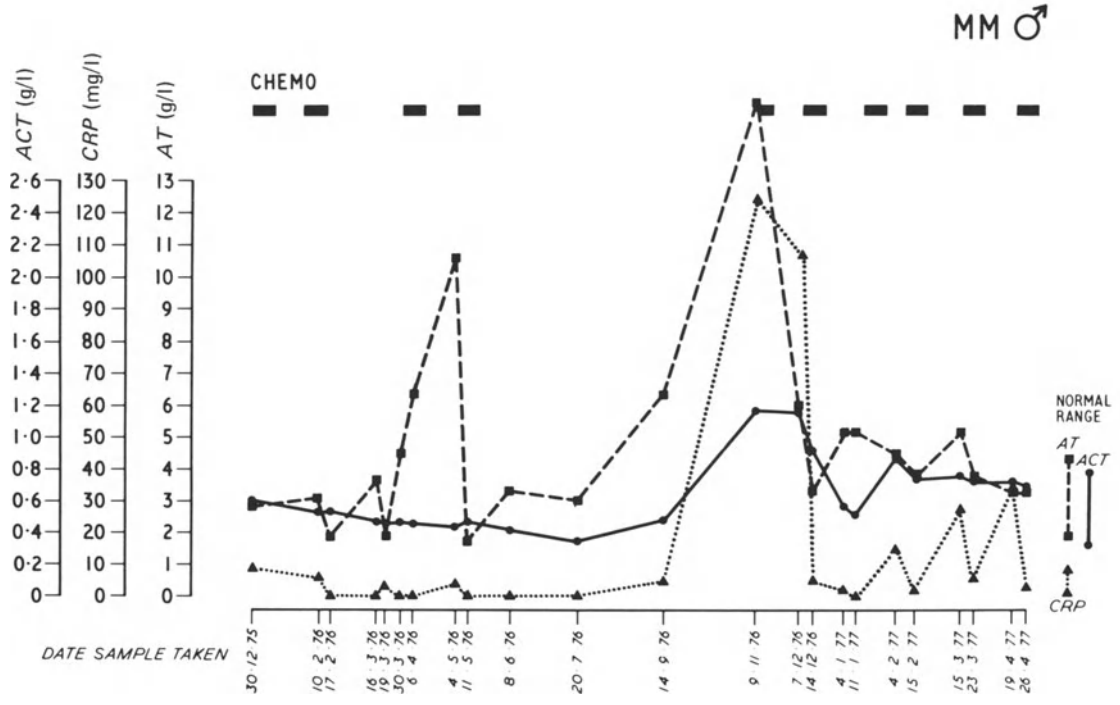

FIGURE 9–3 Changes in APRPs in a 33-year-old man with Hodgkin's disease, diagnosed April 1975; nodal relapse, September 1976, and, subsequently, probable marrow relapse. Increasing lability in the profile reinforces clinical suspicion that the disease is no longer controlled by therapy. ACT, antichymotrypsin; CRP, C-reactive protein; AT, antitrypsin. (From Child et al.,[29] with permission.)

antigens.[17,18] The clinical applications of the measurement of SAA are at present limited. It is not suitable for the diagnosis of secondary amyloidosis, as the protein has the properties of an APRP and will rise in response to many forms of inflammatory stimuli. Raised levels of SAA have been demonstrated in many types of neoplastic diseases, especially when they are metastatic.[116] There have been a few studies of SAA in lymphoid malignancies; the distribution in Rosenthal and Sullivan's[116] investigations is shown in Table 9–5.

From these preliminary data, it appears that SAA can provide a very sensitive general guide to the inflammatory processes associated with neoplasia; however, more detailed studies are needed to be certain whether its high amplitude of response can provide advantages over the simple measurement of CRP.

TABLE 9–6 Diseases in Which Monoclonal Immunoglobulin "Spikes" Are Found in Either Serum or Urine

1. Multiple myeloma with subforms of
   a. Plasma cell leukemia (1%)
   b. Solitary myeloma of skeleton (1%)
   c. Extramedullary soft tissue myeloma (1%)
   Approximate frequency by type of monoclonal protein: IgG 65%; IgA 20%; light chain only 13%; IgD 1%; no paraprotein, IgM, or IgE less than 1%
2. Macroglobulinemia (Waldenström's disease), IgM monoclonal protein (approximately 10% of all monoclonal proteins)
3. Heavy chain disease of gamma, alpha, or mu type; approximate frequency, 0.2%–0.1% of monoclonal proteins
4. Benign monoclonal gammopathy, increasing in frequency with age from 1% to 5% in normal population
5. Papular mucinosis and other rare, nonneoplastic diseases presumably associated with monoclonal antibody formation

From Spiegelberg,[126] with permission.

Serum Immunoglobulins

The identification of monoclonal immunoglobulin "spikes" in serum and urine is an essential step in the analysis and diagnosis of certain diseases of the lymphoid system. The disorders in which such spikes are found are listed in Table 9–6. The measurement of the concentrations of serum polyclonal immunoglobulins is useful in the assessment of humorally mediated immunity. Unfortunately, in many of the earlier studies of immunoglobulin levels in lymphoma, patients had received chemotherapy and radiotherapy prior to the measurements, and the interpretation is equivocal. Earlier work had focused attention on the marked rise of IgE levels[4] and IgD levels[38] in HD, and it appeared the levels of IgG, IgA, and IgM were more likely to be altered by the treatment than the disease.

A major study of 105 patients with untreated

TABLE 9–5 Serum Amyloid A (SAA) Levels in Patients with Leukemia or Lymphomas

Diagnosis	No. of Patients Tested	SAA (ng/dl) Mean	SD	p
Acute myelogenous leukemia	8	3284	2109	0.001
Chronic myelogenous leukemia	9	3666	780	0.001
Acute lymphoblastic leukemia	5	205	19	0.9
Chronic lymphocytic leukemia	12	160	29	1.0
Hodgkin's disease				
Stages I–II	6	150	103	0.9
Stages III–IV	7	1264	876	0.001
Non-Hodgkin's lymphoma				
Stages I–II	3	197	104	0.9
Stages III–IV	12	2175	1451	0.001

From Rosenthal and Sullivan,[116] with permission.
[a] Mean value of each group of patients versus mean value (95 ± 57) of a normal control population of 67 subjects 31–70 years of age. The difference is statistically significant when $p < 0.01$.

HD and 80 with NHL by Amlot and Green showed the variations of immunoglobulin levels.[4] In HD there was a significant increase of IgG and IgE, and in advanced cases the levels of IgM and IgD were subnormal, probably as a result of lymphocyte depletion. In this study there was a reduced level of IgM (in females), IgG, and IgA in NHL; unlike HD, there was no tendency for a high IgE level to be present in the absence of a history of atopy (Figure 9–4).

Rosset et al.,[117] in their study of 132 patients (38 HD, 57 NHL, and 37 chronic lymphocytic leukemia), found that IgG was slightly raised in the early stages of HD and IgM was low in stages II–IV. There was no significant trend in NHL. Chronic lymphocytic leukemia showed the highest incidence of low immunoglobulins. In a smaller group of 27 untreated patients with HD, Douer et al. were unable to demonstrate any significant difference between the levels of IgG and IgM in HD and controls, whereas the IgA level was raised in HD.[42] Both these studies demonstrate that levels of immunoglobulins have a wide variation in health, and in disease the range of levels is extended both above and below the normal range; in some patients the levels are low, whereas others at the same stage have a very high level. This variation of the immunoglobulin levels between patients at each stage of lymphoma is reflected in other studies.[54,134]

In assessing the immunoglobulin levels in lymphoma patients in tropical countries, where particular lymphomas such as Burkitt's lymphoma or lymphomas of intestinal origin are more prevalent, it is important to remember that polyclonal IgG and IgE are common and levels are usually raised

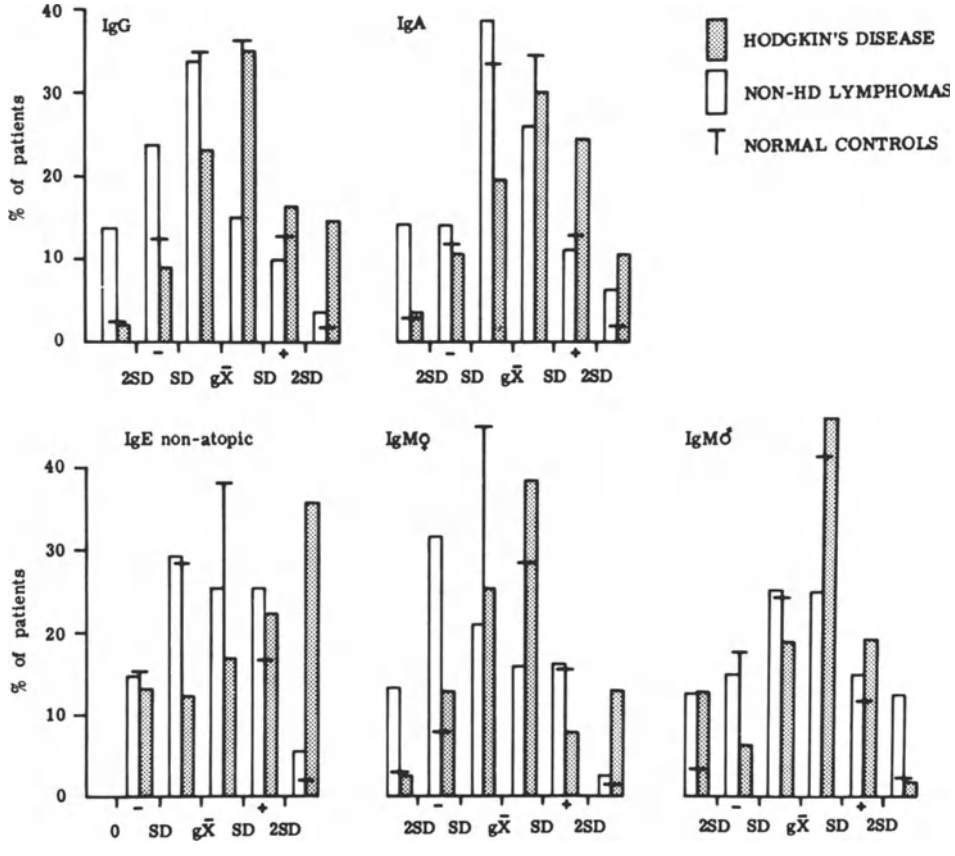

FIGURE 9–4 Distribution of IgG, A, M, and E in lymphomas compared with controls. The histogram demonstrates the percentage of patients falling within increments of 1 SD from the geometric mean ($g\bar{X}$) based on the log-normal distribution of the control population. (From Amlot and Green,[4] with permission.)

as the result of parasitic infection and frequent enteritis.[57] In Bombay, Advani et al. found raised IgG in about 50% of 62 patients with NHL and a high IgA in about 15%.[1]

Splenectomy in normal subjects following trauma is accompanied by reduced serum IgM levels and an increased risk of bacterial infection.[32,43,86] Patients with HD who are splenectomized also have a fall in IgM levels.[43,60,128] However, the increased risk of postoperative infection following splenectomy in HD appears to be related to the underlying neoplastic disease rather than the fall of IgM alone.[28]

Hyperimmunoglobulinemia E

Reagins are the special antibodies that are members of the E class of immunoglobulins. These IgE molecules can become attached to receptors on mast cells and basophilic granulocytes and play a key role in atopic patients. A nonspecific production of hyperimmunoglobulinemia E is a well-recorded feature in several forms of cancer,[114] including the lymphomas.

The detailed studies of Amlot and Slaney of 139 untreated cases of HD has helped to put the topic into perspective:[5] 29 of the patients had evidence of atopy, with raised IgE levels and allergen specificities similar to an atopic but otherwise normal population. Among the nonatopic patients, 76 had normal IgE levels and 44 had raised levels. The incidence of atopy in HD is similar to that in the population in general; the study was unable to find either a cause or effect of the tumor-associated high levels of IgE.

Another large study was reported by Romagnani et al.[112] They evaluated serum IgE levels in 119 untreated and 112 treated patients with HD; 38 of the nonatopic untreated patients showed a significant increase in IgE (>300 IU/1). There was no relationship between the IgE levels and the imbalance of the T lymphocytes with receptors for IgM (Tm) and those with receptors for IgG (Tg). The overall evidence is that the production of IgE is not a consequence of suppressor T cell deficiency. There are several lines of evidence in animals that indicate IgE production is regulated by T lymphocytes.[129] The raised IgE in HD contains both kappa and lambda light chains, indicating it is polyvalent and not from a unique clone of plasma cells.[79] In summary, a high IgE level can occur as a paraneoplastic phenomenon in HD but has no apparent role in the pathogenesis of symptoms.

Waldenström's Macroglobulinemia

Macroglobulinemia as a distinct entity was first described by Waldenström in 1944.[135] The serum electrophoretogram shows a spike of gamma or beta mobility corresponding to a homogeneous monoclonal IgM M component. The main effect of the large amount of IgM is to increase the plasma viscosity, produce multiple hemorrhages and to increase the risk of infection.[46] Sometimes there is hyperglobulinemia due to complexes of monoclonal IgM and IgG which can cause vascular damage.[90] The disease has features that are reminiscent of lymphoid tumors, including enlargement of the lymph nodes, and the marrow and bones are infiltrated with lymphocytoid cells.

Initially, it was thought that Waldenström's disease was a very clear-cut entity, but suspicions that there may be IgM-producing myeloma were aroused by the finding of patients with bone lesions with or without renal disease and a monoclonal IgM; these two features are typical of multiple myeloma but not of Waldenström's disease. IgM myeloma is a rare disease, perhaps constituting a few percent of all multiple myeloma cases. Since the advent of immunologic and physicochemical methods for identifying abnormal M proteins, there have been at least 30 cases of monoclonal IgM plasma cell neoplasia.[142]

Light Chains

Bence Jones proteins are monoclonal light chains. The light chains of immunoglobulins are polyclonal and belong to two main classes: 64% are kappa chains and 36% are lambda light chains.[64] Bence Jones proteinuria occurs frequently in multiple myeloma and in other B cell diseases. The experience of Hobbs shows the typical frequency of this disorder.[64] Bence Jones proteins were present in 69% of 699 cases of multiple myeloma, 60% of 34 with soft tissue plasmacytoma, 15% of 150 cases of chronic lymphocytic leukemia, and 78% of 46 cases of Waldenström's disease; the

incidence in other forms of lymphoma was sporadic. However, with rare exceptions, it is only in multiple myeloma that abundant light chains appear in the urine.

Light chains are freely filtered across the glomerular membrane and are actively absorbed and catabolized by the renal tubular cells.[89] The kidney involvement in multiple myeloma is an important complication, as death can occur as the result of renal failure. The cause of the renal failure is complex: one factor is the nephrotoxic effect of Bence Jones proteinuria; another is the deposition of protein in the renal tubules. Hypercalcemia and dehydration are frequent precipitating causes of uremia in the patient[39] who already has a compromised kidney with tubular nephropathy and interstitial nephritis.[13] The renal glomerular damage associated with light chains in multiple myeloma and, more rarely, in other NHL and chronic lymphocytic leukemia can be assessed by the measurement of serum creatinine, urea, or the glomerular filtration rate.

Renal tubular damage can be demonstrated by increased amounts of low molecular weight proteins in the urine (<70,000 daltons), as the proximal renal tubule is the normal site of catabolism of these proteins; the small proteins such as β_2-M, retinol-binding protein, and insulin are almost totally removed from the glomerular filtrate as it passes through the proximal tubules. The tubular proteinuria can be shown by either polyacrylamide gel electrophoresis[131] of concentrated urine or measurement of the concentration of an appropriate low molecular weight indicator protein.[11] The relationship between the amount of light chains in the urine in multiple myeloma and the excretion of retinol-binding protein (molecular weight 21,200 daltons) is shown in Figure 9–5.

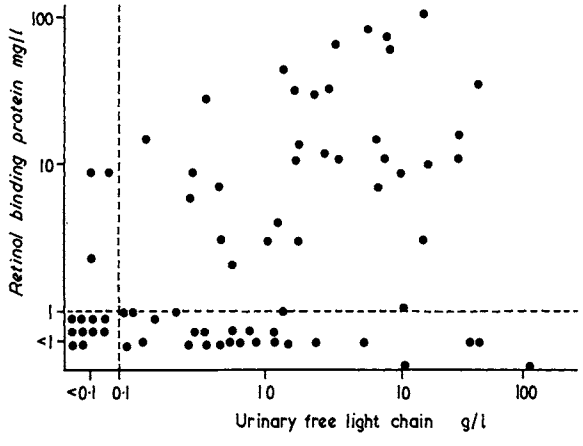

FIGURE 9–5 Relationship of urinary light chain excretion and retinol-binding protein levels in multiple myeloma (C.M. MacLennan and E.H. Cooper, unpublished data). Notice that several of the patients who excrete light chains do not have any increased excretion of retinol-binding protein; this suggests that these light chains do not interfere with the tubular reabsorption of this low-molecular-weight protein.

Whereas these proximal tubular defects of protein reabsorption are related to the light chain load, it has been reported that some patients can develop renal failure without producing excess light chains,[62,140] and some patients have massive filtered loads of light chains that are not nephrotoxic (see Figure 9–5). By contrast, in the nephropathy of Waldenström's disease the M component in the blood is directly deposited in the glomerular capillary luminae, forming thrombi.[96]

Polymerization of Paraprotein

Approximately 20% of cases of myeloma are of the IgA class.[63] This class of monoclonal immunoglobulins has the property of forming polymers in the blood, so that it can occur as the monomer, dimer, trimer, quadramer, etc. Polymeric IgA differs from monomeric IgA in that the polymeric variety has a single joining (J) chain,[59,76] a small polypeptide chain of 15,000 daltons bound to the heavy chain of the dimer.[76] It is thought that it is the amount of intracellular J chain that controls whether the IgA is secreted as a monomer or polymerized.

In normal plasma 5%–15% of IgA is polymeric, whereas in secretions the predominant form is the dimer. Roberts-Thompson et al., in a study of 25 patients with IgA myeloma, found 14 were mainly monomeric (monomer > 50%) and 11 polymeric (monomer < 50%); within each group there was wide variation, but predominantly polymerized forms were only a feature of multiple myeloma.[111] This preliminary study drew attention to the relationship of large amounts of polymeric IgA and the development of the hyperviscosity syndrome.[81] This syndrome is characterized by a grossly elevated relative serum viscosity, bleeding, retinopathy, and a wide spectrum of neurologic lesions;[111] it is most often encountered in Waldenström's disease. In an additional study using refined techniques,[27] it was shown that marked hyperviscosity occurred in 5% of 179 patients with

IgA myeloma, and that polymerization and viscosity were related. However, it seems that it is the aggregation of IgA that produces the clinical symptoms.

Hyperviscosity can also occur in IgG myeloma; in this case it is due to either a concentration-dependent aggregation of the paraprotein or molecular asymmetry.[52] IgA tends to bind to other proteins, in particular, albumin.[52] Recently it was shown that α_1-microglobulin, a low molecular weight serum protein (33,000 daltons),[19] is strongly bound by monoclonal monomeric IgA in multiple myeloma; therefore, the serum α_1-microglobulin levels in patients with a normal glomerular filtration rate tend to reflect the amount of IgA monomer (Cooper EH, Forbes M, and MacLennan I, unpublished data).

Lymphadenopathies Associated with Dysgammaglobulinemia

The association between enlarged lymph nodes and dysgammaglobulinemia is well established in a spectrum of malignant and benign diseases. These disorders were reviewed by Pruzanski,[109] who classified them as shown in Table 9–7. It can be seen that they include diseases in which the dysgammaglobulinemia is a key factor in the immunopathology, for example, Bence Jones proteinuria nephropathy, IgM M component and hyperviscosity syndromes, amyloidosis, and those diseases in which the formation of immune complexes can result in tissue damage, such as systemic lupus erythematosus. However, apart from infections and immunization-/ and drug-induced conditions, the precise interrelationships of the lymphadenopathy and the production of an abnormal gamma globulin profile is uncertain.

TABLE 9–7 Lymphadenopathy Associated with Dysgammaglobulinemia

Chronic lymphadenopathy with systemic manifestations
Angioimmunoblastic lymphadenopathy
(Immunoblastic lymphadenopathy)
Giant lymph node hyperplasia (Castleman's disease)
Sinus histiocytosis with massive lymphadenopathy
Chronic benign lymphadenopathy in childhood
Malignant diseases
Waldenström's macroglobulinemia and related syndromes
Plasmacytic dyscrasia (including multiple myeloma, plasma cell leukemia)
Amyloidosis
Gamma heavy chain disease
Alpha heavy chain disease
Mu heavy chain disease
Hodgkin's disease
Lennert's lymphoma
Other lymphomas
Leukemias
Kaposi's sarcoma
Collagen–vascular diseases
Juvenile rheumatoid arthritis
Rheumatoid arthritis
Felty's syndrome
Systemic lupus erythematosus
Mixed connective tissue disease
Lymphoid granulomatous vasculitis
Sjögren's syndrome
Peetoom-Meltzer syndrome
Other disorders
Drug-induced lymphadenopathy
Infectious diseases
Sarcoidosis
Vaccination and immunization
Primary dysgammaglobulinemia I
Graft-versus-host reaction

From Pruzanski,[109] with permission.

Less Common Disorders of the Plasma Proteins in Lymphoma

Monoclonal gammopathy occurs in 6%–8% of patients with diffuse lymphomas, but only in 1% of those with nodular lymphomas.[93] The monoclonal spike is less common in HD. Other expressions of immune disorders in HD are the rare occurrence of Coombs'-positive autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura.[84] The literature on this unusual complication has been reviewed by Jones[70] and Waddell and Cimo.[132]

Complement

Several investigators have studied complement levels in HD. The earlier reports did not find any relationship between the levels and the incidence of relapse or remission.[85] Later it was shown that total hemolytic complement and complement levels were elevated in relapse and untreated disease,[22,133,139] and levels of complement were shown to be related to those of other APRPs.[139]

The levels of circulating immune complexes were reported by Amlot et al.[6] to be associated with generalized symptoms in HD, but this has not been confirmed by others.[24,73] Recent studies using the Raji cell assay in children with HD indicate a progressive rise in circulating immune complexes related to stage; levels are normal or very low in patients with complete remission.[22]

Ferritin

Ferritin is the soluble iron-storage protein found in cells of the body. The protein consists of an apoprotein shell of 440,000–600,000 daltons and an iron core containing 2500–4000 iron atoms. The administration of iron to the whole animal or to cells is a stimulus for the synthesis of the apoprotein.[61,141] The level of serum ferritin is raised in HD and NHL.[105] In a study of 125 untreated patients with HD, there was a tendency for the serum ferritin to rise with stage and to be high when there were symptoms, while the serum iron and transferrin levels were low.[68] This reaction is considered to be a nonspecific response of the reticuloendothelial system to the malignant process. It is of interest that a similar rise in serum ferritin is found in rheumatoid arthritis (Figure 9–6), and both rheumatoid arthritis and advanced HD share the common property of being strong stimuli for the elevation of CRP and SAA.

Detailed studies of ferritin have shown there are about 20 human isoferritins derived from two or three common subtypes: H is characteristic of the acid isoferritins in the heart, HL is common to the heart and liver, and L is the more basic ferritin of the liver and spleen.[44] Cancer cells tend to contain ferritins that are more acidic than those normally in the human liver; as yet there is no evidence as to whether there is a significant change in the plasma isoferritin composition in HD.

β_2-Microglobulin

The β_2-M originates from nucleated cells, where it forms the light chain of HLA. The lymphoid tissue in health appears to be one of the major sources of this protein in the plasma. In common with all low molecular weight proteins, this protein (11,800 daltons) is freely filtered by the glomeruli and normally over 99% is reabsorbed and catabolized by the cells lining the proximal tubules. The normal range of serum levels is 0.4–2.4 mg/l, which tends to rise to an upper limit of 3.0–3.5 mg/l in people over 70 years of age. The protein turnover is very fast, with a serum half-life of less than 1 hour. Elevations of serum β_2-M can occur in a variety of malignant neoplasms, especially lymphoproliferative tumors, and certain benign diseases (mainly autoimmune disorders); levels always rise passively and in proportion to any de-

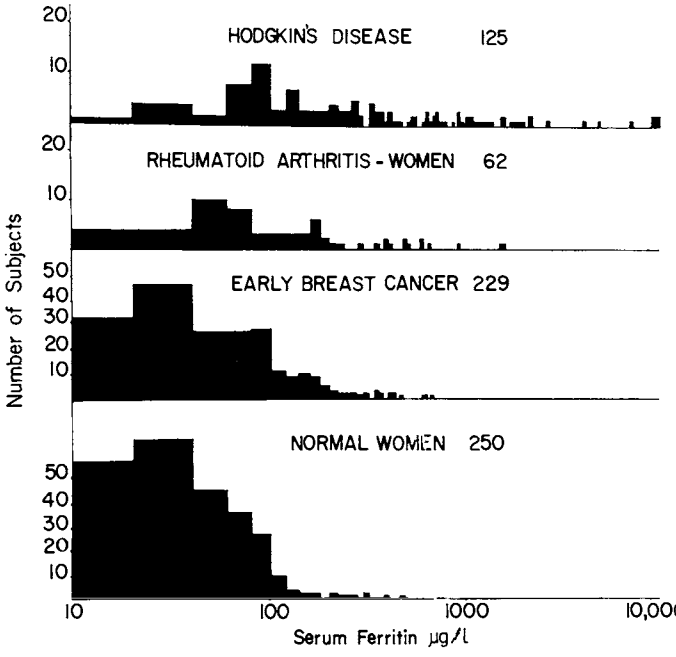

FIGURE 9–6 Distribution of serum ferritin concentration (log scale) in normal women and patients with breast cancer, rheumatoid arthritis, and Hodgkin's disease. (From Jacobs et al.,[68] with permission.)

TABLE 9–8 Distribution of β_2-microglobulin in Untreated Chronic Lymphocytic Leukemia Staged According to the Rai Classification

	Rai Stage		
	0	I–II	III–IV
Number	21	13	8
Inter-quartile range	2.0–2.8	2.7–5.3	4.4–16.9
Median	2.6	3.0	7.5
95% non-parametric confidence limit	2.0–2.8	2.6–4.7	3.0–24.2

[a] From Späti et al.,[125] with permission.

crease in the glomerular filtration rate.[35,72,127] The levels of β_2-M are unrelated to those of APRPs, although coincidental elevations may occur; β_2-M levels are unaffected by trauma and little affected by sepsis.

Levels in Chronic Lymphocytic Leukemia

Despite its nonspecific character, the serum β_2-M level appears to have potential as a marker of tumor burden in chronic lymphocytic leukemia[124,125] and multiple myeloma.[100] The disturbance of serum β_2-M levels in HD and NHL is more complex and has less immediate clinical application as a routine marker. In untreated chronic lymphocytic leukemia, the level of β_2-M is related to tumor burden (Table 9–8). This general relationship has been confirmed in other studies of untreated and treated patients.[124] Longitudinal studies of the general slope of the rate of change of serum β_2-M levels show the way in which the level tends to rise slowly in patients whose disease is poorly controlled, whereas the level is fairly stable in patients whose disease is intrinsically nonprogressive (Figure 9–7). It is of interest that despite the rise in serum β_2-M levels in stages II–IV the amount of chronic lymphocytic leukemia in lymphocytes is less than that in normal peripheral blood lymphocytes.[108] In practical terms, the slow rate of change of this marker suggests that measurements every 4 months are adequate to give warning of an insidious expansion of the tumor burden. Serum β_2-M measurements are most helpful in assessing a new case of chronic lymphocytic leukemia or monitoring patients who are being watched without treatment.

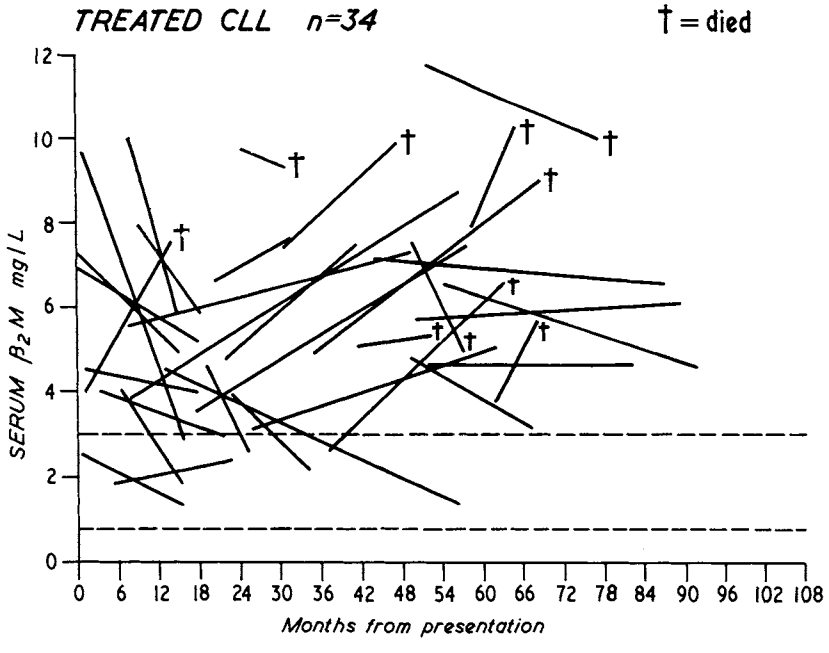

FIGURE 9–7 Slopes indicating the overall rates of change of serum β_2M levels in patients with stage II–IV chronic lymphocytic leukemia; levels tend to fall during the first 12–18 months as a result of chemotherapy; later, a long, slow phase of rise occurs until the patient's death. In contrast, patients with stages O and I who do not require treatment tend to have levels that are either within the normal range or rarely above 4 mg/l and remain constant for many years. (From Späti et al.,[125] with permission.)

Levels in Hodgkin's Disease and Non-Hodgkin's Lymphoma

In HD the levels of β_2-M tend to increase with stage. In Amlot and Adinolfi's series a β_2-M level greater than 3 mg/l was found in 7 (33%) of 22 patients with stages I and II, and in 40 (71%) of 56 with stages III and IV.[3] A similar trend was observed in our data: 5% of 38 patients with stages I and II and 43% of 23 patients with stages III and IV had a serum β_2-M level greater than 3 mg/l; whereas only 3.8% of 104 patients in complete remission from stages III and IV had a raised serum β_2-M.[33]

The distribution of β_2-M in NHL is illustrated by data from a multicenter trial organized in Leeds (Table 9–9).[33] The overall analysis of NHL, excluding Burkitt's lymphoma, showed that 31% of stage I and II patients and 61% of stage III and IV patients had β_2-M levels of over 3 mg/1. When this set of patients was subdivided into those whose histology (according to Rappaports classification) was known to be associated with a good prognosis (NWDL; NPDL; NM; DWDL), the initial pretreatment level of β_2-M was not associated with survival. In contrast, among the group of patients with a histology known to be associated with a poor prognosis (DM, DH, DPDL and unclassified), those patients with an initial β_2-M level of over 4 mg/1 had a projected median survival of 10 months, compared to 60 months in those whose level was under 4 mg/1. Further studies are needed to see if this marker continues to make this prognostic stratification in the poor prognosis group. The biologic explanation for this phenomenon is not known.[33] The time course of β_2-M and CRP levels in illustrative cases of lymphoma are shown in Figures 9–8 and 9–9.

Levels in Multiple Myeloma

The early reports of serum β_2-M levels in disease showed that multiple myeloma was frequently associated with high levels. Levels over 3 mg/1 were reported by Shuster et al.[123] in 39% of 103 patients and by Kin et al.[74] in 58% of 36 Japanese patients. This observation has since been confirmed by several other studies. In our initial experience with 36 patients, pretreatment levels were over 4 mg/1 in 16 and under 4 mg/1 in 20. This level of β_2-M was shown to be significantly related to survival: the median survival for the two groups was 15 months and 46 months, respectively. This has been confirmed in an independent study of 90 patients in Manchester: the median survival in the group with initial β_2-M levels over 4 mg/1 was 15 months, whereas it was 38 months in the group with β_2-M levels under 4 mg/1 (Scarffe H and Forbes M, personal communication, 1982). In our own and the Manchester series it was shown that the pretreatment serum β_2-M level is a powerful predictor of survival and retains this effect when all the usual criteria of estimating tumor burden, as described by Durie and Salmon,[45] are taken into account. A study of 78 patients in France

TABLE 9–9 Percentage Distribution of Serum β_2-Microglobulin in Untreated Non-Hodgkin's Lymphomas

Histologic Type[a]	No. of Patients	β_2-Microglobulin <3.0 mg/l	3.1–5.0 mg/l	>5.0 mg/l
NWDL, NPDL, NM	43	63	23	14
DWDL	19	26	42	37
DPDL	27	74	18	9
DM	20	20	30	50
DH	25	36	36	28
Unclassified	16	43	43	14
African Burkitt's lymphoma[b]	33	27	52	21

From Cooper and Child,[33] with permission.

[a] NWDL, nodular well differentiated lymphocytic; NPDL, nodular, poorly differentiated lymphocytic; NM, nodular mixed; DWDL, diffuse well differentiated lymphocytic; DPDL, diffuse poorly differentiated lymphocytic; DM, diffuse mixed; DH, diffuse histiocytic.

[b] Data from Cooper et al.[37]

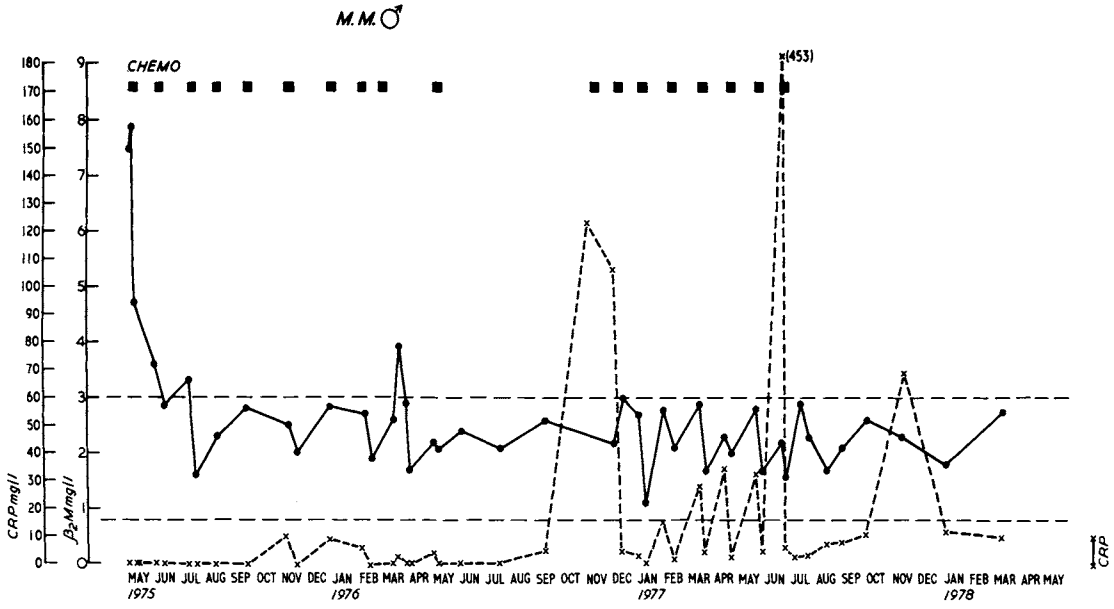
M.M. ♂
CHEMO
(453)
CRP mg/l
β2M mg/l
CRP
MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC JAN FEB MAR APR MAY
1975
1976
1977
1978

FIGURE 9–8 Independence of the CRP response and β_2M levels in a 32-year-old man with Hodgkin's disease. (Same patient as in Figure 9–3.) (From Child et al.,[29] with permission.)

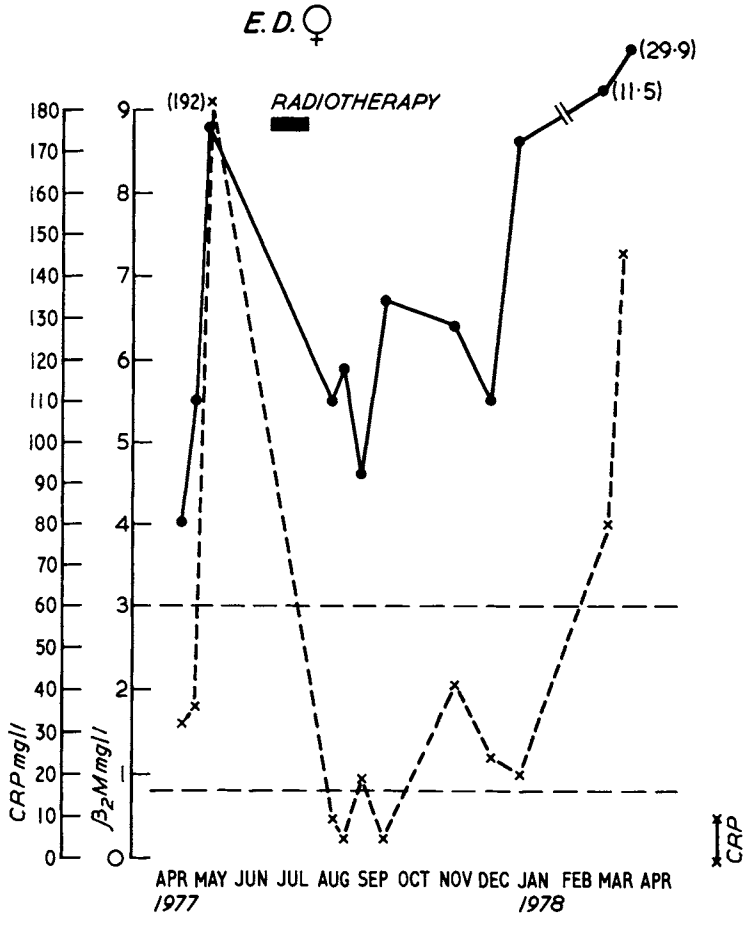

FIGURE 9–9 Independence of the CRP and β_2M levels in a 71-year-old woman with Hodgkin's disease complicated by diabetes and an un-united fracture of the right femur in relation to Paget's disease. Staging procedures were limited and local radiotherapy was given to the neck and axilla in July 1977. On reevaluation in March 1978 there was hepatosplenomegaly and a trephine biopsy showed evidence of HD.

has confirmed the high correlation of the total-body burden to multiple myeloma cells and the serum β_2-M level.[12] Examples of the evolution of serum β_2-M levels and the main clinical events in two cases of multiple myeloma are shown in Figures 9–10 and 9–11. The overall trends of β_2-M in patients who died within 2 years (Figure 9–12) are contrasted with those in patients who survived more than 2 years (Figure 9–13).

A recent Japanese study indicated that low levels of serum β_2-M in multiple myeloma are significantly associated with certain IgG heavy chain allotypes.[99] On the other hand, the distribution of raised and normal levels of β_2-M are not significantly different in the lambda and kappa types of IgA and IgG myelomas. The concentration of β_2-M shows a general tendency to rise in parallel with the concentration of paraprotein, but there is considerable variation between patients within the same class of paraprotein.

The levels of serum β_2-M are infrequently raised in patients with benign gammopathy,[14,94] but the overlap with the levels in the less aggressive forms of multiple myeloma make it impractical to use this measurement to distinguish these two conditions.[12,95] On the other hand, the diagnostic criteria for these two disorders are different.[87] Morell and Riesen[94] found that a quantitative immunohistochemical examination of the bone marrow and the percentage M component in the total serum protein separated the benign from malignant disease in about 90% of cases in their series.

In summary, current evidence suggests that a raised serum β_2-M can occur in most forms of malignant lymphoproliferative disease and tends to be related to the tumor mass. The protein has a very short half-life, so that a marked increase in its production rate is needed to sustain a general rise in its level. All classes of proliferating lymphocytes, including T and null cells, have been shown to be capable of producing a raised serum β_2-M, but there is often a wide variation in the membrane turnover rate between different clones. Infectious mononucleosis is an example of a benign lymphoproliferative disorder in which the β_2-M level is raised coincidentally with the production of the proliferating atypical mononuclear cells,[37,67,123] which are known to be of T cell origin.[122] It was

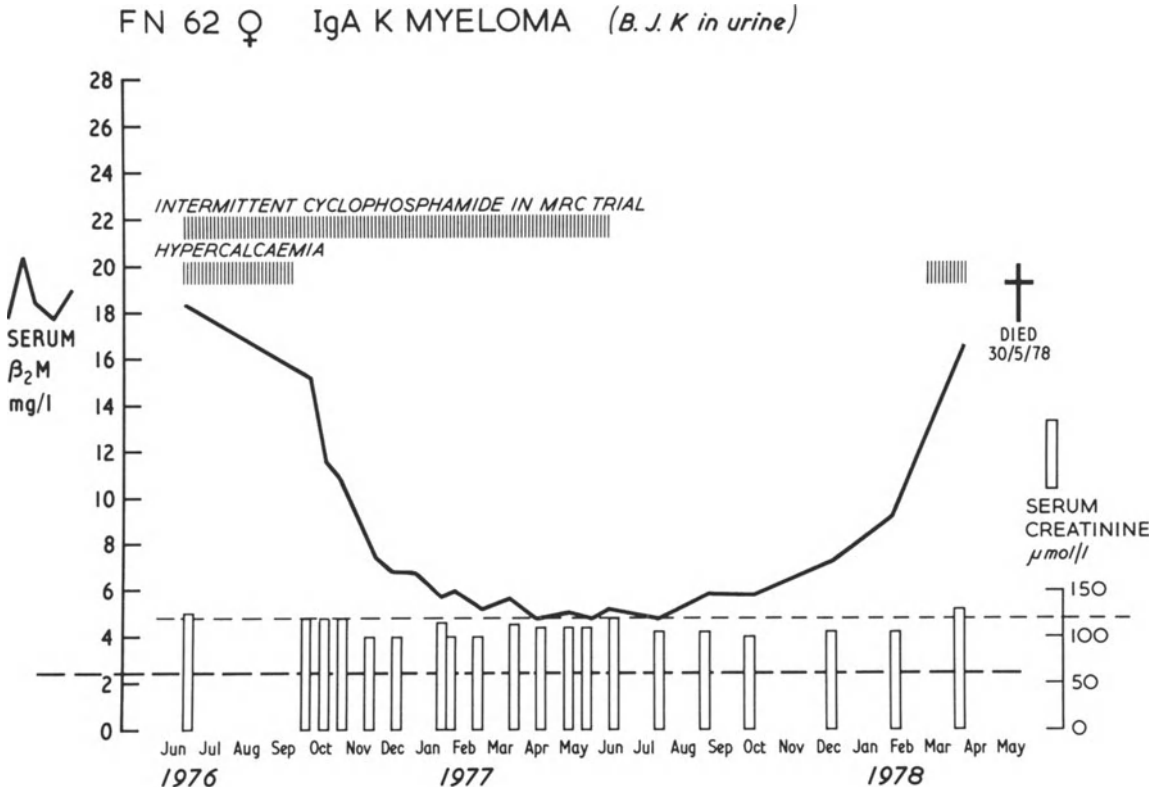

FIGURE 9–10 Elevation of serum creatinine and β_2M levels in a 62-year-old woman with IgA K multiple myeloma and hypercalcemia. (Patient under care of Dr. J.A. Child.)

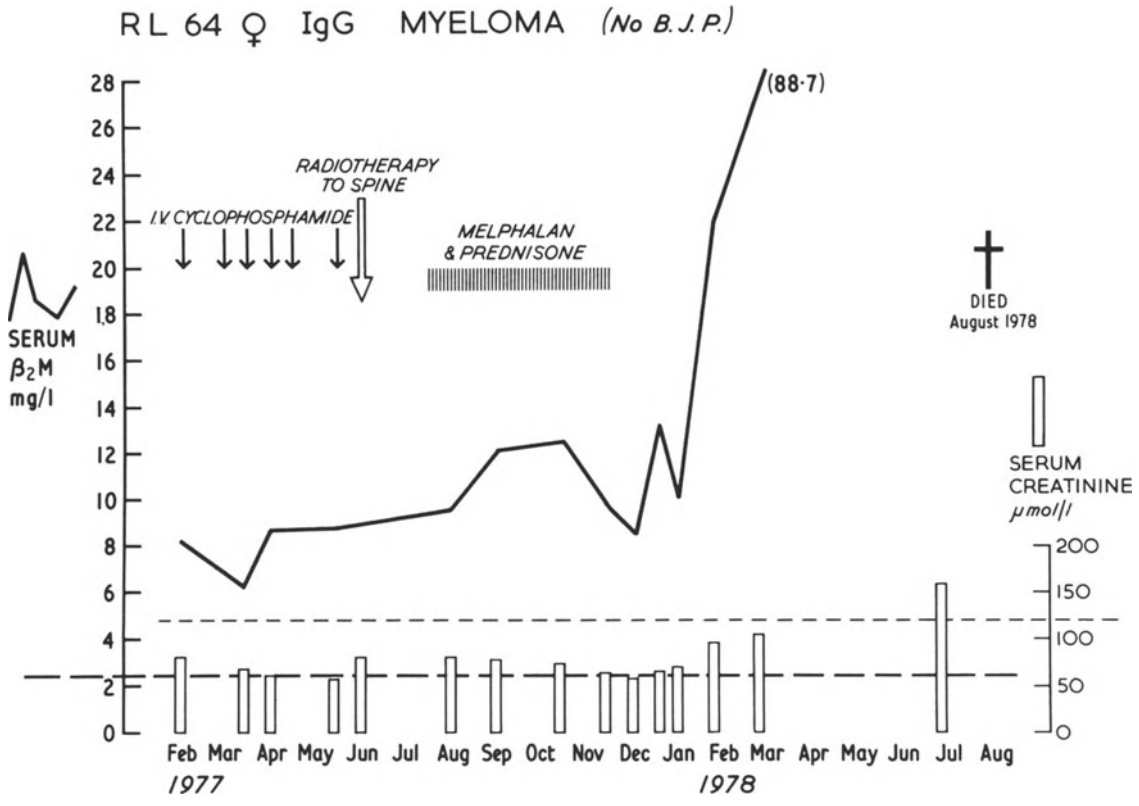

FIGURE 9–11 Elevation of serum creatinine and β_2M levels in a 64-year-old woman with an aggressive case of IgG multiple myeloma that failed to respond to chemotherapy. The IgG last value is associated with a coincidental uremia.

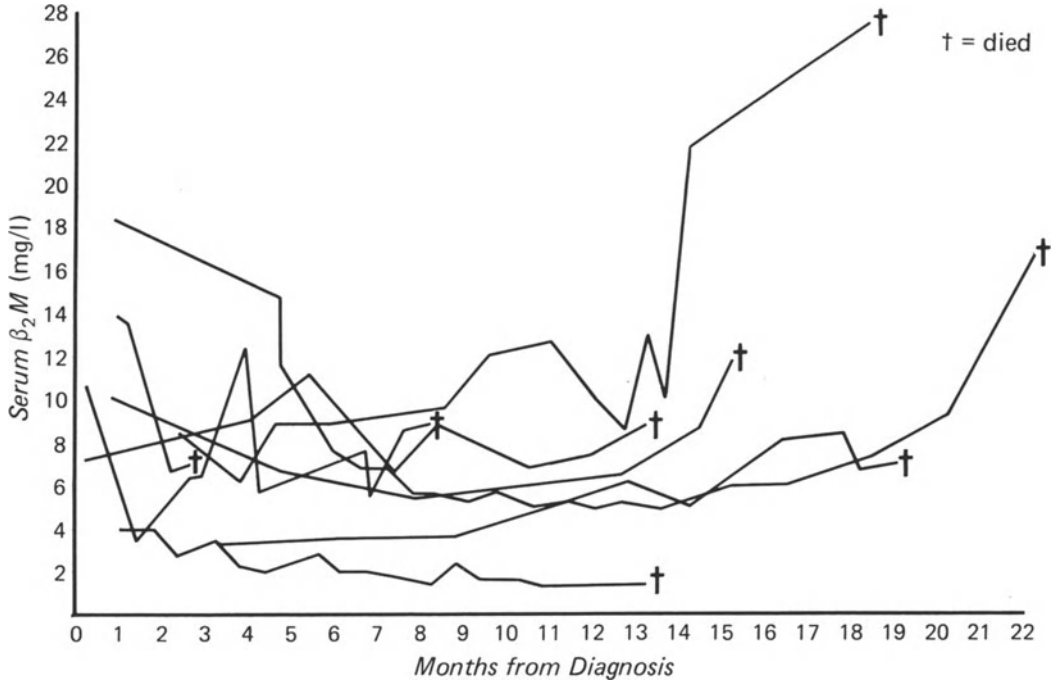

FIGURE 9–12 Evolution of serum β_2M in patients who died within 24 months of presentation. (From Norfolk et al.,[100] with permission.)

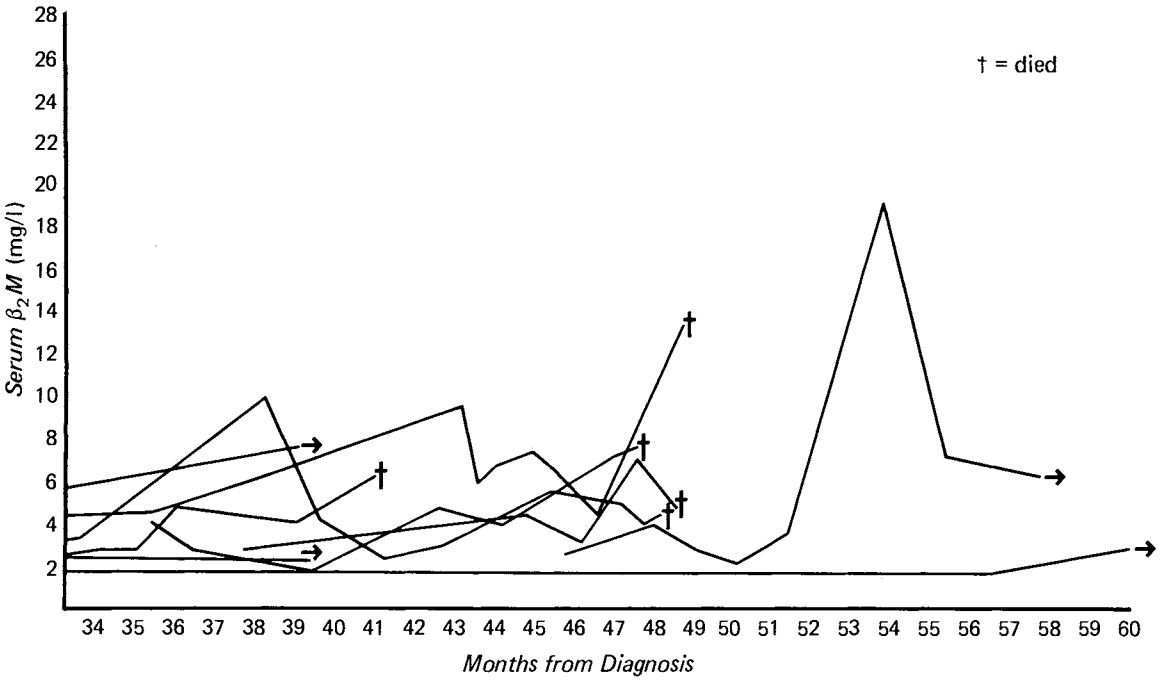

FIGURE 9–13 Evolution of serum β_2M in patients who survived 40 or more months. (From Norfolk et al.[100])

recently shown that high serum β_2-M levels are frequent in myeloproliferative disorders and various types of ineffective erythropoiesis, such as megaloblastic anemias due to B_{12} or folate deficiency, and homozygous thalassemia (Norfolk D, Child AJ, and Cooper EH, unpublished data).

Immunologic Abnormalities

As each new technique for the exploration of the function of the lymphoid system has been devised, investigators have been quick to see if they can show another defect in lymphocyte function in Hodgkin's disease. The methods currently used for investigating T cell function are listed in Table 9–10. Consequently, the literature now contains a plethora of accounts of these abnormalities, mainly in HD but with some studies of NHL. It is now evident that B cell function is unaffected by HD alone, but can be depressed by a combination of splenectomy, chemotherapy, and radiotherapy; T cell function, however, is abnormal in all patients with the disease. The recent reviews of DeVita and Hellman[40] and Schulof et al.[120] help bring the evidence into their clinical context.

TABLE 9–10 Tests of T Cell Function

Delayed hypersensitivity response
T cell proportions and absolute numbers
T cell subset proportions
Phytohemagglutinin reactivity
T cell colony formation in agar
Concanavalin A–induced suppressor cell activity
T cell–mediated spontaneous and antibody-dependent cytotoxicity
T cell chemotaxis

Adapted from Schulof et al.,[120] with permission. (See Schulof et al. for the technical details of these tests.)

The delayed hypersensitivity reactions are generally depressed and the absolute numbers of circulating T cells tend to be low in at least one-half of patients with HD. The more recent tests of T cell function tend to show that properties such as chemotaxis, T cell colony formation, and spontaneous and antibody-dependent cytotoxicity are abnormal in HD.[120] It is of interest that a study of 43 Egyptians with HD and NHL showed a pattern of defective cellular immunity similar to that in Americans[46] and comparable results were found in Indians with NHL in Bombay.[1]

In multiple myeloma and macroglobulinemia there is an increased susceptibility to bacterial infection.[47,118] Unlike the lymphomas, in these disease it is the primary response to antigenic challenge that is impaired, whereas secondary re-

sponses and delayed hypersensitivity reactions are less impaired.[118] The main defect in multiple myeloma is the inability of myeloma B cells to differentiate into plasma cells and secrete immunoglobulin in response to antigens or mitogens. It now appears that myeloma B cells can proliferate *in vitro* in response to pokeweed mitogen in the presence of irradiated T cells, but they do not produce immunoglobulin. Hence the defect in differentiation appears to be beyond the point of DNA synthesis.[57] The focus has now moved to the immunoregulatory function of the T cells in multiple myeloma.[23,103,104] The helper T cell function is intact for polyclonal immunoglobulin secretion, but the suppressor T cells do not function effectively due to a deficit in the nonsuppressor T cell subset.[102] It has been suggested that this deficit could be a compensatory response to either the monoclonal proliferation or the involvement of immunoregulatory T cells in the pathogenesis of multiple myeloma.[102] Several tests can be combined to give a "lymphocyte score," which has proved to be a good prognostic indicator in HD.[20]

There is unequivocal evidence that T lymphocyte function is altered in lymphoid disease. The part played by the humoral environment in bringing about the immunodeficiency states, however, is less well understood. The levels of copper[80] and zinc can influence cell-mediated immunity; delayed hypersensitivity reactions can be restored in zinc-deficient children by giving them this trace metal.[55] CRP[98,106] and AGP[31] have both been shown to modify lymphocyte function *in vitro.* Recently it was shown that SAA exerts an immunosuppressive effect in the early antibody response of mice to sheep red blood cells.[18] These serum proteins and trace metals are usually abnormal in widespread lymphomas and may play a part in amplifying the immunodeficiency, but it is unlikely that they play a significant role in the disturbances of immunity that are well known in stage I HD.

Serum Enzymes

During the 1960s and early 1970s there has been a general interest in serum enzymes as possible aids to cancer diagnosis and monitoring. The discovery that the relative frequency of various isoenzymes (identified by their electrophoretic mobility) of an enzyme such as lactate dehydrogenase (LDH) can be disturbed in cancer prompted considerable research, but little of this information has been useful in routine practice. Serum LDH levels have been shown to be proportional to the tumor mass and predict prognosis in American and African Burkitt's tumor.[8,9] In a study of 30 patients with diffuse histiocytic lymphoma, Schneider et al. observed a highly significant difference between the survival of patients with LDH below 500 units and the survival of those with an LDH greater than 500 units; the survival rates were 67% and 13%, respectively.[119]

Liver Function Tests

Jaundice is usually a late complication of lymphomas; usually it is due to a compression of the biliary tract, but it can also be caused by the cholestatic action of certain drugs. Serum albumin levels tend to be well maintained until there is a massive tumor burden in lymphoma or widespread multiple myeloma. A comparison of the liver biopsies obtained at staging laparotomy and the levels of serum enzymes reflecting parenchymal cell function was made by Belliveau et al.[15] The γ-glutamyltransferase levels were raised in patients with biopsies showing cholestasis and hepatitis, or granulomas; but less than one-half of those with Hodgkin's tissue in the liver biopsy had increased levels. Alkaline phosphatase tended to be raised in these three forms of liver pathology. The tendency for alkaline phosphatase to rise progressively with stage in HD had been recorded in a previous study by Aisenberg et al.[2]

A study in our laboratory[110] of serum γ-glutamyltranspeptidase in routine hematologic practice showed that high levels (> 100 units/l) occurred in 6 (12%) of 50 HD patients; all 6 had stage III or IV disease. High levels were also found in 7 (27%) of 26 NHL patients, 4 of whom had very aggressive disease. Polychemotherapy was not a cause of high levels of γ-glutamyltranspeptidase. In multiple myeloma 4 (18%) of 22 patients had high levels; 2 of these patients had advanced disease with considerable numbers of circulating plasma cells. This study reconfirmed the fact that although abnormal liver function tests are a sign of advanced disease, they add little to the assessment of the prognosis based on staging procedures and clinical examination; yet the liver function test is asked for very often by oncologists, many of whom take little notice of the result.

Conclusions

As yet there is no universal system for the monitoring of lymphomas and multiple myeloma. Many patients have the good fortune to be treated by highly experienced, competent physicians at all stages of their illness. Often, however, during the middle of the course of a disease that may last several years, patients are cared for by less experienced doctors and an early recurrence can be missed (especially in NHL, for which therapy is still far from ideal). The clinical chemistry laboratory can help the less experienced doctor by drawing attention to evidence of disease when the patient is apparently in remission. CRP, ceruloplasmin, and β_2-M seem to be the plasma proteins to monitor because they can give warning signals, but the sensitivity of A A protein may eventually make it a vital part of any protein profile in lymphoid malignancy. Whatever is chosen as the marker system, it is clear that serial measurement is the key to appreciating the course of a malignant disease of the lymphoid system in the individual patient.

It is becoming evident that biochemical tests can help in the stratification of some forms of NHL and multiple myeloma. Time will tell whether some form of biochemical stratification, albeit used retrospectively in the final analysis, can help in the testing of novel approaches to therapy. We wait for new, simple, and effective cellular immunology tests to be developed to help in the selection of therapy that is optimal for the particular patient.

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CHAPTER 10

Hematologic Aspects of Malignant Lymphoproliferative Disease

SANFORD JAY KEMPIN

Malignant lymphoproliferative disorders, resulting from the monoclonal proliferation of lymphocytes, influence both hematopoiesis and hemostasis. These effects may be caused by the presence of the tumor itself, or through secondary properties of the tumor such as immunoglobulin secretion or release of tissue thromboplastin-like material. Apart from these direct influences, treatment-induced alteration by chemotherapy or radiotherapy may result in profound, often prolonged, and occasionally fatal hematologic complications. Although many of these complications may be seen in patients with solid tumors, the central role of the lymphocyte in the development of normal hematopoiesis and immune competence (both of which appear to be essential in the protection against malignant disease) underlines the complexity of these alterations and the difficulties encountered in their therapy. This chapter is devoted to the role of lymphoid cells in normal hematopoiesis and alterations produced by their malignant proliferation and treatment.

Role of Lymphoid Cells in the Development of Normal Hematopoiesis

All mature hematopoietic cells (erythrocytes, neutrophils, basophils, eosinophils, platelets, monocytes, and lymphocytes) are ultimately derived from embryonic mesenchymal tissue giving rise to hematopoietic stem cells. The initial stimulus for a marrow stem cell to begin its terminal differentiation is not known, however, subsequent steps are more clearly understood. The latter occur under the influence of colony-stimulating factors (CSF), which are themselves derived from hematopoietic cells[67,68,209,282,320,343] and other tissues,[109,168,339] as well as other hematopoietins.[50,124,176,318] Once the cell has achieved a morphologically recognizable state, the differentiation pathway is irreversible. Although lymphocytes are derived from marrow progenitor cells, their terminal differentiation, unlike that of other hematopoietic cells, occurs outside the bone marrow under the influence of the thymus (T lymphocytes) or the bursa equivalent (B lymphocytes). Lymphocytes in turn influence erythro-, myelo-, and monopoiesis (Figure 10–1). Recent evidence suggests that T lymphocytes may even influence hematopoietic stem cell function.[342] At the present time there is no evidence that lymphocytes directly affect thrombopoiesis.

Erythropoiesis

The pronormoblast, the earliest morphologically recognizable red cell precursor, has already undergone a number of maturational steps under the influence of erythropoietin, secreted by the kidney. T cells play a contributory role in the proliferation of early red cell precursors.[68,236,320] The nature of this interaction is known (secretion of CSF), and, in a canine model, thymocyte-stimulated erythropoiesis *in vivo* has been reported.[68] A malignant counterpart of normal T cells has also been found to possess erythroid-potentiating activity.[125] Recently, subpopulations of T lymphocytes, defined by monoclonal antibodies, have been demonstrated to inhibit or stimulate the growth of red cell pre-

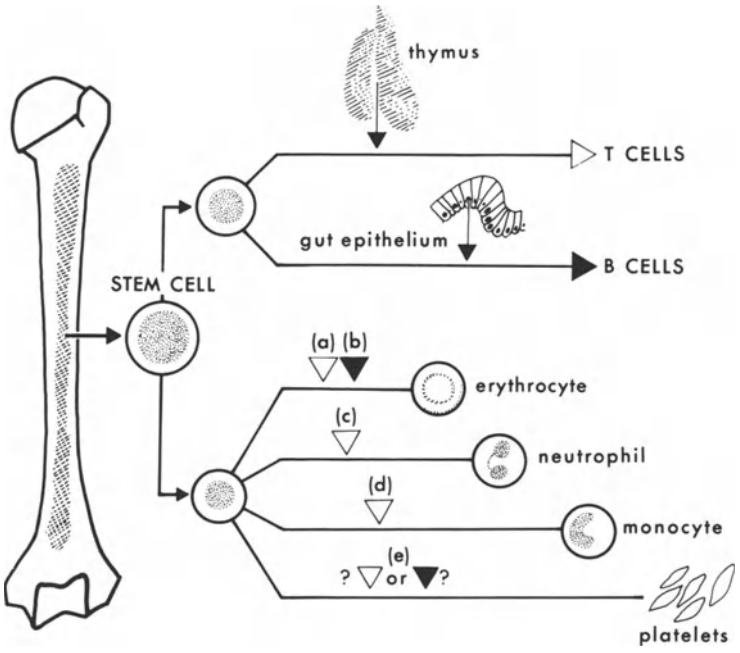


FIGURE 10–1 Role of lymphoid cells in the development of normal hematopoiesis. (a) T cells stimulate or inhibit normal red blood cell precursors. (b) B cells, likewise, play a stimulatory or inhibitory role in red cell production. (c) T cells, by secreting CSF, and by interacting with other hematopoietic cells, influence the production of neutrophils. In disease, inhibitory influences occur. (d) A T lymphocyte growth factor for monocytes and macrophages derived from a cell line has been described. (e) There is no evidence at this time that either B or T lymphocytes influence megakaryocytopoiesis.

TABLE 10–1 Median Hematologic Values at Diagnosis in Patients with Malignant Lymphoproliferative Disease

Disease[a]	Bone Marrow Involvement (%)	Hemoglobin (g/dl)	Leukocytes ($\times 10^9$/l)	Platelets ($\times 10^9$/l)
CLL	100	(55)[b]	(17)[c]	(39)[d]
DWDL	100	14 (43)	6 (14)	207 (17)
Myeloma	100	(70)	6.5[e] (16)	195[e] (12)
Waldenstrom's macroglobulinemia	100	(60–80)	(13)	(33)
NPDL	62	14 (26)	6.5 (12)	246 (4)
DPDL	34	13 (38)	7.9 (14)	268 (12)
DHL	15	13 (36)	7.3 (4)	304 (0)
Hodgkin's disease	7	11.9–13.0[f]	8.6–13.9[f]	

Data from

Alexanian R, Balcerzak S, Bonnet JD, Gehan EA, Haut A, Hewelett JS, Monto RW: Prognostic factors in multiple myeloma. Cancer 36:1192–1201, 1975

Bloomfield CD, McKenna RW, Brunning RD: Significance of haematological parameters in the non-Hodgkin's malignant lymphomas. Br J Haematol 32:41–46, 1976

Boggs DR, Sofferman SA, Wintrobe MM, Cartwright GE: Factors influencing the duration of survival of patients with chronic lymphocytic leukemia. Am J Med 40:243–254, 1966

Cline MJ, Solomon A, Berlin NI, Fahey JL: Anemia in macroglobulinemia. Am J Med 34:213–220, 1963

Imhof JW, Baars H, Verloop MC: Clinical and hematological aspects of macroglobulinemia Waldenstrom. Acta Med Scand 163:349–366, 1959

Kaplan H: Hodgkin's Disease. Cambridge, Mass, Harvard University Press, 1980

Kyle RA, Bayrd ED: The Monoclonal Gammopathies. Multiple Myeloma and Related Plasma-Cell Disorders. Springfield, Ill, Thomas, 1976.

[a] CLL, chronic lymphocytic leukemia; DWDL, diffuse, well-differentiated lymphocytic lymphoma; NPDL, nodular, poorly differentiated lymphocytic lymphoma; DPDL, diffuse, poorly differentiated lymphocylic lymphoma; DHL, diffuse histolocytic lymphoma.

[b] Percentage anemic.

[c] Percentage neutropenic.

[d] Percentage thrombocytopenic.

[e] Mean value.

[f] Mean value, depending on stage (see Kaplan[172]).

cursors or burst forming units (BFU-E).[320] Cells inhibiting erythropoiesis *in vitro* have been reported in T cell chronic lymphocytic leukemias (CLL).[154,234] Some workers have not found an obligatory role of lymphocytes in BFU-E growth;[343] therefore, the *in vivo* relevance of these observations is unclear. Decreased, normal, and increased numbers of T lymphocytes have been observed in the lymphoproliferative disorders, however, anemia or polycythemia related to these quantitative abnormalities alone has not been described. The role of B lymphocytes in normal hematopoiesis has not been defined. Both B lymphocytes, through secretion of an inhibitor of erythropoiesis,[188,189,205] and thymic tissue[153] have been implicated in the syndrome of red cell aplasia (see below).

Myelopoiesis and Monopoiesis

The major proliferative stimuli for myelopoiesis are the CSFs. These are produced by monocytes and macrophages,[68] stimulated T lymphocytes,[67] and a number of tissues, both normal[109] and malignant.[339] Inhibitors of normal granulocytic stem cells, but not CSF, are present in lymphoid leukemic cells and are at least partly responsible for the neutropenia seen in these patients.[46] An unusual disorder, "T cell lymphocytosis with neutropenia," has been described[8] in which a proliferation of cytotoxic suppressor T cells proliferate with myeloid maturation arrest. Eosinopoiesis is less well characterized. However, sensitized lymphocytes are capable of stimulating eosinophil colony growth[282] and neonatal thymectomy diminishes antigen-mediated eosinophilia.[222] Eosinophilia is not uncommon in the lymphoproliferative disorders,[315] and it is conceivable that either the malignant proliferation or the body's response may be responsible for the increased production of these cells. Monocyte production appears to be under the control of specific CSFs secreted either by monocytes themselves[68] or by T lymphocytes. A human T lymphocyte–derived growth factor for myeloid and macrophage colonies has been described[209] in a malignant cell line. Monocytosis is not infrequently observed in Hodgkin's disease,[172] but whether the malignant cell is secreting a monocyte CSF is not known. Various hematologic abnormalities are seen in the malignant lymphomas. Hematologic values reported in lymphoproliferative diseases are summarized in Table 10–1.

Hematologic Disorders in Malignant Lymphoma

Anemia

A decreased red cell mass is a common observation in patients with lymphoproliferative disorders.[28] Although the majority of lymphoma patients at diagnosis will be found to have a hgb level above 12gm/dl—nearly 45% will become anemic during the course of the disease.[274] There are many etiologies (Table 10–2) and it is not uncommon to find multiple causes in one individual.

Decreased Production

Replacement of the marrow cavity by malignant lymphocytes is not quite an adequate explanation of decreased production. In a number of lymphoproliferative states in which marrow involvement is common (CLL, multiple myeloma, nodular lymphomas) anemia is only seen in advanced stages, particularly if immunohemolytic anemia and hypersplenism are absent. In CLL—in which even at early stages (stages 0–II) the marrow is nearly replaced by small B lymphocytes—anemia, by definition, is absent. In nodular lymphomas—in which the incidence of marrow involvement may be as high as 80%[56,259,270]—anemia is uncommon. Distortion of marrow architecture by myelofibrosis has been described in Hodgkin's disease[201,239] and hairy cell leukemia[35] to a degree that would seriously impair hematopoiesis.

Immune-mediated inhibition of erythropoiesis may be secondary to an immunoglobin, generally IgG, directed against erythropoietin[186] or red cell precursors.[188,189] Pure red cell aplasia has been described in CLL[1] with a cyclophosphamide-induced remission resulting in normal erythropoiesis, myelopoiesis, and thrombopoiesis, and no objective evidence of CLL. T cell or thymus-derived inhibitors of erythropoiesis have been described in T cell leukemias[154,234] and thymomas,[11,103,113] and a marrow-suppressive factor has been described in the serum of a patient with Hodgkin's disease.[103] Each of these inhibitory states may result in the syndrome of red cell aplasia.[153,186,187] Treatment of the underlying dis-

TABLE 10–2 Etiology of Anemia in the Lymphoproliferative Disorders

Decreased Production
Tumor replacement
Immune-mediated inhibition
Treatment-related inhibition
Evolution into leukemia
Nutrient deficiencies (iron, folic acid, vitamin B_{12}, protein)
Anemia of chronic disease
Increased Destruction
Nutrient deficiencies
Enzymatic disorders
Anemia of chronic disease
Intramedullary destruction
Drug-induced hemolytic anemia
Autoimmune hemolytic anemia
Microangiopathic hemolytic anemia
Hypersplenism
Bleeding

order, the use of corticosteroids[106] and immunosuppressives,[188,341] occasionally splenectomy,[92] plasmapheresis,[225] and the resection of a thymoma,[11] if present, may reverse these abnormalities.

Chemotherapy and radiotherapy are important causes of anemia due to decreased production. Most drugs used in the treatment of lymphomas influence normal hematopoiesis either by inhibiting or stimulating marrow precursor cells. Early erythroid precursors are particularly susceptible to these agents; megaloblastic changes in the bone marrow erythroid pool, a result of decreased DNA synthesis, are the earliest morphologic changes. Because of the long life span of the mature red blood cell and the intermittent nature of most lymphoma treatment regimens, significant anemia directly related to chemotherapy alone is rarely a problem. Most often the combined effects of chronic disease, infection, uremia, and radiotherapy play a more important role than chemotherapy. Consequently, neutropenia and thrombocytopenia are, clinically, the most important problems related to the administration of these drugs. Antimetabolites (methotrexate, 6-mercaptopurine, cytosine arabinoside, azauridine), alkylating agents (nitrogen mustard, chlorambucil, cyclophosphamide), intercalating agents (anthracyclines), and vinca alkaloids all inhibit erythropoiesis (as well as myelo- and thrombopoiesis). These are reversible changes. However, cumulative injury to marrow stem cell precursors may occur with alkylating agents, and, although overall marrow cellularity appears normal, marrow reserves may be impaired.[228]

The toxicity from radiotherapy is directly related to the dosage received by the exposed tissues.[312] The presumed abscopal effect of therapy[31] is thought to result from injury to circulating stem cells. As with chemotherapy, the toxic effects are usually reversible but they may be more prolonged.[180–182,312] The incidence of leukemia in patients treated with alkylating agents and with combined modality approaches is significant,[53,71,72] and in this setting refractory anemia is often the initial sign of a leukemia evolution. Bone marrow alterations, including dyserythropoiesis, sideroblastic anemia, and increased blast forms, should alert the physician to this complication. Remissions are unusual and brief. Only symptomatic therapy (transfusional therapy, antibiotics) is administered until remission induction therapy becomes absolutely necessary.

Iron is necessary for normal erythropoiesis. In patients with lymphoma of the stomach,[114] intermittent gastrointestinal blood losses may result in severe iron deficiency. Surgical treatments such as partial gastrectomy,[137] complete gastrectomy,[2,310] and gastroenterostomies[32]—may themselves cause iron deficiency as a result of achlorhydria[231,310] or a loss of other gastric secretions[310] by bypassing the duodenum (the main site of iron absorption) and by blood loss at anastomotic sites. Involvement of the small bowel by

lymphoma may induce a malabsorption state[145,161,304] as well as bleeding, with the subsequent development of iron, folic acid,[256] and B_{12} deficiency (the B_{12} deficiency could also result from total gastrectomy because of the absence of intrinsic factor). This syndrome is particularly frequent in immunoproliferative small intestinal disease (alpha-chain disease or "Mediterranean lymphoma"),[13,91,264,294] but has also been found in other non-Hodgkin's lymphomas,[91,304] as well as lymphomas seen in association with celiac sprue and dermatitis herpetiformis.[16,112] These deficiencies may result in megaloblastosis and iron deficiency with hypochromia. Furthermore, amyloid deposits in the stomach and small intestine,[38,39,165] radiotherapy, and intestinal resections[32] may all result in an inability to absorb hematopoietically necessary nutrients and result in severe anemias. Protein malabsorption may cause impaired erythrocyte production,[104] and subsequently an anemia that is normocytic and normochromic.

All lymphoproliferative disorders may result in a hypoproliferative anemia that is not clearly related to nutritional deficiencies, chemotherapy, nor the presence of lymphoma in the marrow; rather, it is related to the presence of a chronic debilitating illness in which infectious complications and chronic inflammation play a significant role. This disorder, known as the anemia of chronic disease,[52] is perhaps the commonest form of anemia in patients with underlying malignancy, including lymphomas, and is the most difficult to understand and treat. The degree of anemia roughly correlates with the severity, but not the duration of the underlying disorder. The anemia is usually normocytic and normochromic, but occasionally microcytic and hypochromic (the latter not as severe as in iron deficiency anemia). A normal or low reticulocyte count is present, a reflection of both decreased erthropoiesis and possibly a "compensated" anemic state. The serum iron is low,[131] and the iron-binding capacity and transferrin saturation are all decreased. Reticuloendothelial iron is normal or increased,[131,226] however, the serum ferritin is elevated or normal[203]—a useful distinguishing feature from iron deficiency, in which it is low. The pathogenesis of this disorder includes a decreased erythrocyte life span,[159,322,323] impaired marrow erythropoiesis,[269] and defective iron utilization.[143] This defective utilization results from the release of leukocyte endogenous mediator,[171] and lactoferrin,[325,326] and macrophage ingestion of the lactoferrin–iron complex.[326] As this form of anemia is generally seen in uncontrolled malignancy, and because the patient generally does not have symptoms of the anemia, blood transfusions are given only if absolutely necessary and iron is withheld unless a deficiency can be documented.

The treatment of these hypoproliferative states includes nutritional supplementation with iron, folic acid, or B_{12} if deficiencies exist and are irreversible. The underlying lymphoma should be treated with appropriate therapy. Nonspecific hematinic cocktails are ineffective unless, fortuitously, they happen to treat an unrecognized deficiency.

Increased Destruction

The life span of red blood cells is approximately 120 days. Premature destruction results in hemolytic anemia and a subsequent marrow response resulting in reticulocytosis. Clinically, the patient presents with symptoms of weakness and signs of jaundice. Splenomegaly, hepatomegaly, lymphadenopathy and edema may be present, depending upon the type and extent of the lymphoproliferative disorder. Laboratory evaluation demonstrates reticulocytosis, indirect hyperbilirubinemia, increased lactate dehydrogenase and, if the disorder is immune in nature, a positive Coombs' antiglobulin test. Mild degrees of destruction may not be reflected in the above findings, but become apparent on red cell survival studies. Because decreased production is common in these disorders, the absence of reticulocytosis does not eliminate hemolysis as a cause for the anemia. Almost all hemolytic anemias in the lymphoproliferative disorders result from extrinsic destruction. Exceptions include iron deficiency,[86] pernicious anemia,[302] and enzymatic abnormalities[323] in which intrinsic defects result in decreased survival. Decreased red cell survival, particularly in widespread metastatic disease, has been described in breast and lung cancer[21] and might be seen in lymphomas.

Most red cell destruction occurs outside the bone marrow; however, intramedullary destruction may occur through erythrophagocytosis in Hodgkin's disease.[183] This is an essential aspect of a rare and often fatal disorder, histiocytic medullary reticulocytosis,[328] which is now recognized as a disorder of the monocyte–macrophage lineage.

Once red blood cells are released into the circulation, the major causes of increased destruction are immunohemolytic anemia and hypersplenism. The production of antibodies directed against a red cell membrane antigen, although uncommon, represents one of the most serious complications of these disorders as well as a therapeutic challenge.[193] Although most of the immunohemolytic anemias are associated with an imbalance in the immune system related to the underlying disease or treatment, drug-induced hemolytic disease must always be considered. Drugs commonly associated with this effect are listed in Table 10–3. Recently, VM-26 (Teniposide) has been associated with an IgG antibody and hemolytic anemia.[134] The incidence of a Coombs' antiglobulin test (not necessarily synonomous with hemolytic anemia) differs with each disorder with the highest incidences in CLL[244,255,311] and γ heavy chain disease.[194,336] Approximately 25%–40% of all patients diagnosed as having autoimmune hemolytic anemia ultimately are found to be suffering from a lymphoma; the anemia may antedate the diagnosis[241] or complicate the disease.[227]

Autoimmune hemolytic anemias are defined by the avidity of the antibody for the red cell antigen at various temperatures (cold versus warm reacting). Most of these anemias are due to warm-reacting antibodies that bind best to red cells at 37°C. The majority of these antibodies are IgG.[77] The predominant subclass is IgG1, with IgG2–4 considerably less common.[96] Some of these antibodies have Rh specificity,[77,96] such as anti-c and anti-e, and occasionally other defined antibodies such as anti-LW, anti-U, and anti-i.[96] More commonly they function as panagglutinins without defined specificity. Red cell destruction is a result of the interaction of the antibody (Fc portion) and the monocyte–macrophage system, primarily through the interaction of the latter's Fc and less through C3b receptors.[111] Clearance is extravascular, primarily in the spleen.[111] Complement fixation depends upon the type of immunoglobulin (greater with IgM than IgG) and the density and type of IgG molecules on the red cell surface.[289] Because IgG1 and IgG3 are efficient fixers of complement, and monocytes have receptors for the Fc portion of IgG1 and IgG3, these two subclasses are most active in causing hemolysis. The Coombs' antiglobulin test is the laboratory correlate of this reaction and approximately 20% of patients with a positive direct test have IgG, 30% have IgG and complement, and 50% have complement alone on the surface of the red blood cell.[101,338] In Hodgkin's disease, autoimmune hemolytic anemia generally occurs late in the course of the disease,[94,199] although it has rarely occurred as an initial manifestation.[36,199] It is felt to be a reflection of active and advanced disease.[199] In CLL, autoimmune hemolytic anemia occurs spontaneously, however, rare instances of treatment-related hemolysis have been described after irradiation.[202] This may be related to the sensitivity of T suppressor subsets to radiotherapy or chemotherapy.

TABLE 10–3 Drugs Implicated in Immunohemolytic Anemia

Antimicrobial Agents
p-Aminosalicyclic acid
Cephalosporin derivatives
Isoniazid
Penicillin
Streptomycin
Sulfonamides
Miscellaneous Agents
α-Methyldopa
Chlorpromazine
Levodopa
Quinidine
Thiazides
VM-26 (Teniposide)

The treatment of this form of hemolysis consists of corticosteroids, generally prednisone, at a dose of 40 mg/m^2 daily, as well as treatment of the underlying lymphoproliferative disorder. Treatment with steroids may need to be prolonged as long as 3 months, during which gradual tapering is undertaken.[230] Approximately 80% of patients respond.[230] The mechanisms of the steroid action include decreased antibody production,[247] decreased antibody avidity for the red cell antigen,[275] and a change in monocyte–macrophage clearance of antibody-coated red blood cells by an alteration of monocyte complement receptors.[290] Splenectomy is recommended in those patients who are unresponsive to steroids or who develop significant complications with steroid administration.

Radionuclide sequestration studies may be helpful in the decision to undertake splenectomy.[62,123] Splenectomy is most likely to be successful if the antibody is of the IgG rather than the IgM type, since splenectomy only affects the clearance of the former[111]—hence the absence of

treatment effect in autoimmune hemolytic anemia with cold-reacting antibodies (see below), which are principally IgM. Cytotoxic drugs, particularly cyclophosphamide, are included in most lymphoma treatment regimens and contribute to the control of this problem.[230,247] These agents do not alter monocyte–macrophage clearance, but work through an inhibition of antibody production—hence the lag time between their institution and treatment effect. Because steroids and cytotoxic agents are now generally applied after diagnosis in most patients with lymphoma, splenectomy is rarely necessary.

Cold-reactive antibodies (those most active below 37°C) are rare causes of autoimmune hemolytic anemia in the lymphoproliferative disorders. Most of these cases result from the idiopathic cold agglutinin syndrome, although there are reports of its presence in Waldenström's macroglobulinemia, CLL, non-Hodgkin's lymphoma,[260,291,313,338] and, in one case, a poorly defined lymphoproliferative disorder.[174] Most of these antibodies are directed against anti-I or anti-i, and are IgM in type. They appear to share idiotypic specificity,[260] and the monoclonal types have kappa light chains.[139] The antibodies fix complement, and C3b-mediated phagocytic sequestration,[197] occurring primarily in the Kupfer cells of the liver,[111] is responsible for most red cell destruction. This reaction is mediated in part by C3b inactivator, which may cause the release and recirculation of these IgM–C3b coated erythrocytes.[111] Intravascular hemolysis may result if the thermal amplitude of the cold agglutinin is broad. Both polymorphonuclear leukocytes[261] and platelets[329] may also bind this cold agglutinin and undergo destruction.

The clinical syndrome includes acrocyanosis, anemia, and jaundice. The age distribution is similar for the idiopathic condition and those associated with lymphoproliferative disorders (sixth to eighth decade).[291] Infection, by stimulating macrophage function,[334] may imbalance a "compensated" state and is often a trigger for a hemolytic episode. The direct antiglobulin test performed at 37° Centigrade is generally positive for complement and negative for immunoglobulin. Treatment is directed at the underlying lymphoproliferative disorder. The treatment of the idiopathic syndrome consists of raising the ambient temperature, protection against cold, and vigorous treatment of infections. Splenectomy, steroids, and immunosuppressive agents are generally not helpful, although plasmapheresis, by removing IgM (predominantly intravascular), may have some therapeutic benefit.[313]

The spleen is an organ of particular interest to the physician treating lymphoproliferative disorders from the points of view of diagnosis, staging, as well as pathologic states resulting in hypersplenism. In the normal individual approximately 20% of the total red cell mass and 30% of the platelet mass are concentrated in the spleen;[18] whereas in an individual with an enlarged spleen nearly 50%–90% of the platelet mass may be sequestered in this organ.[18] In cancer patients decreased survival of red blood cells may be due to hypersplenism, despite the absence of clinically apparent splenomegaly.[322] All lymphoproliferative disorders may present with splenomegaly, but this is not an isolated phenomenan except in an unusual form of lymphoma, primary splenic lymphoma.[5,80,303] The incidence of splenomegaly at diagnosis in the various disorders varies considerably in different series and is probably a reflection of the different staging procedures used.[122,212,274] Approximately 30% of nonpalpable spleens are involved with lymphoma when a surgically staged group of non-Hodgkin's lymphoma patients is studied.[56,270] At autopsy the incidence of splenic involvement is approximately 40%,[272] which represents a composite of both treatment effects and progression of disease. Occasionally, splenomegaly may be seen without splenic involvement or with only minimal involvement. The latter may result in splenomegaly as a consequence of CSF production, with subsequent myeloid metaplasia.[129].

The diagnosis of hypersplenism is based upon the presence of pan- or isolated cytopenia, and a cellular bone marrow in a patient with an enlarged spleen. If immunocytopenic states and bleeding can be ruled out, the diagnosis of hypersplenism is likely. The decision to perform a splenectomy is based in large measure on clinical judgment. Labeled red cell sequestration studies may be helpful.[62] Bone marrow involvement which appears to preclude normal hematopoiesis is not necessarily a contraindication of splenectomy.[4,279]

It is unusual for hypersplenism to be present at diagnosis of a lymphoproliferative disorder. Most often this complication is observed as a late manifestation of disease, generally in heavily pretreated patients, in whom the enlarged spleen was resistant to conventional chemotherapy and radiotherapy. Hypersplenism is unusual in Hodg-

kin's disease, mainly because of the application of splenectomy as part of staging laparotomies at diagnosis. In CLL an enlarged spleen is common but usually quite sensitive to chemotherapy, at least initially, and blood counts in this disorder improve rapidly once an alkylating agent–prednisone combination is begun. For advanced CLL, splenectomy[279] as well as splenic irradiation[49,87] may be useful as palliative measures. In prolymphocytic leukemia, splenomegaly and resultant hypersplenism are initial manifestations.[54] Recent results of aggressive chemotherapy suggest this as the treatment of choice,[316] although splenectomy may be quite helpful in selected cases. Chemotherapy is usually effective in the non-Hodgkin's lymphomas, and splenectomy is done only as a palliative measure late in the course of the disease. Splenomegaly is seen in almost 90% of patients with hairy cell leukemia at presentation.[35] Splenectomy is the treatment of choice for severe pancytopenia,[35] although reports of effective chemotherapy may alter the therapeutic approach in the future.[82,126] Splenic rupture is an unusual complication of lymphoproliferative disorders,[22,273] but it must be diagnosed promptly and treated surgically.

Because the spleen is an integral part of the mononuclear–phagocyte system,[48] as well as a source of antibody production,[3,278] splenectomy results in a number of changes in circulating blood cells as well as alterations in immune competence. Morphologic changes in the red blood cells are the most striking effect of splenectomy. Poikilocytosis, anisocytosis, nucleated red blood cells, reticulocytosis, target cells, fragmented cells, and Howell-Jolly bodies are present.[204,301] Leukocytosis and thrombocytosis accompany these erythrocyte alterations but are generally transient.[221,301,337] The thrombocytosis occasionally results in thromboembolic disease,[152] but rarely bleeding,[144] as in essential thrombocythemia or chronic myelogenous leukemia. Sternberg-Reed cells have occasionally been observed in the peripheral blood of Hodgkin's disease patients, whether splenectomized or not.[34]

The earliest report of infectious complications related to splenectomy was published in 1952.[177] Since that time a burgeoning literature has appeared that confirms this initial observation,[26,190,300,331] particularly in the pediatric age group; in this age group an incidence of 5.4% mortality from sepsis has been reported, particularly when an underlying disorder was present or chemotherapy was administered.[300] Infectious complications are seen particularly in children under 1 year of age;[300] this may be related to the increase in upper respiratory tract infections observed in this age group and the absence of prior natural immunity. The infections encountered tend to be more overwhelming[26,331] and cause a higher mortality rate[84,300] than in nonsplenectomized individuals. Recent studies demonstrate that even splenic irradiation may cause a significant decrease in splenic function.[73]

The risk of severe infection in lymphoma patients having undergone a splenectomy ranges from 1.5% to 10%,[84,307] with an overall mortality of 0.5%.[84] The risk appears to be greatest within 1 year after splenectomy.[190,300] *Streptoccus pneumoniae, Neisseria meninigitidis, Escherichia coli,* and *Hemophilus influenzae* are the most common infecting organisms.[300] Disseminated intravascular coagulation is not uncommon in splenectomized patients with overwhelming sepsis;[26] it is related to thromboplastin generation by endotoxin-exposed leukocytes,[237] platelet activation by endotoxin,[268] and the lack of clearance by the spleen of activated clotting factors.[296] Antibody production is impaired in Hodgkin's disease patients after therapy,[297] and patients undergoing splenectomy for staging should be vaccinated with pneumococcal vaccine prior to the procedure. It is important to remember that when the spleen is removed in the other lymphoproliferative disorders, the same problems as seen in Hodgkin's disease patients may be present, and presurgical vaccination as well as rapid treatment of infection in these patients is important. Partial splenectomy has been proposed as an alternative staging procedure to prevent these complications,[308] however, the possibility of inadequate pathologic staging must be considered if this is undertaken.

Neutrophil Disorders

Normal neutrophil numbers and function are required for optimal infection fighting capability. Neutropenia is an unusual observation at initial diagnosis in the lymphoproliferative disorders. One exception to this is hairy cell leukemia (leukemic reticuloendotheliosis): leukopenia (predominantly neutropenia) has been observed in 41%–57% of patients.[35,54,126] In CLL, although the relative percentage of neutrophils is decreased, the

absolute number is generally above 10^9l. Cyclic neutropenia has been reported in patients with lymphoproliferative disorders independent of chemotherapy.[235,285] Neutropenia, however, is commonly observed after chemotherapy and is often the limiting factor in the administration of these agents. Because of the short life span of the mature neutrophil, neutropenia secondary to treatment is more marked than the decrease in red blood cells (due to the long survival of the mature erythrocyte).

Because cell-cycle–specific and cell-cycle–nonspecific agents are administered, the kinetic alterations in neutrophil productions are complex.[130] Nitrosoureas may cause prolonged and even biphasic marrow toxicity, with nadir counts occurring after 1 month. This is observed after BCNU,[340] an agent that affects both cycling and noncycling cells and is active in both Hodgkin's and non-Hodgkin's lymphomas. Patients receiving long-term alkylating agent therapy have been shown to have decreased marrow reserve.[75,130] The stem cell population as well as the microenvironment of the marrow is damaged after chemotherapy[228] as well as radiotherapy. The latter alters neutrophil production; recovery depends on the extent of the marrow irradiated and the total dosage received.[180-182,312] An unusual cyclical oscillation of neutrophils after 3 years of chemotherapy was observed in a patient with multiple myeloma.[59] Autoimmune neutropenia has been reported in a case of T-cell lymphoma.[191] In addition to chemotherapy and radiotherapy, a number of drugs used commonly in the treatment of lymphoma patients have been associated with agranulocytosis (Table 10–4). These agents are unpredictable in their efforts and may cause either abrupt-onset or delayed-onset neutropenia. The latter agents (i.e., phenothiazines) generally cause marrow suppression, whereas antimicrobials are responsible for an antibody-mediated agranulocytosis. Neutropenia not directly related to chemotherapy, radiotherapy or hypersplenism (rare) must be considered drug induced, and a careful medication review should be undertaken and any drug reported to induce neutropenia should be discontinued.

Qualitative abnormalities of neutrophil function have been observed, related both to the therapy and the underlying disease.[58] Neutrophils demonstrate inhibition of phagocytosis after irradiation.[295] Folic acid antagonists,[81,254] purine,[254] and pyrimidine analogues[81] inhibit the microbicidal killing capacity of neutrophils. Resting and stimulated neutrophil hexose monophosphate shunt activity, required for the detoxification of hydrogen peroxide and regeneration of reduced pyrimidine nucleotides, is decreased by cyclophosphamide,[254] as is *in vitro* candidacidal activity.[81] The latter is also inhibited by cytosine arabinoside, methotrexate, daunorubicin, doxorubicin, and actinomycin D.[81] Both vinblastine and vincristine inhibit resting and stimulated hexose monophosphate shunt activity.[254] In addition, they interfere with the microtubular structure of leukocytes.[215] Vinblastine precipitates the filament bundles of tubulin in neutrophils.[314]

Glucocorticoids are commonly used in the lymphoproliferative disorders and influence hematopoietic cells, particularly leukocytes, in numerous ways. These compounds cause a neutrophilic leu-

TABLE 10–4 Drugs Associated with Neutropenia and Thrombocytopenia

Anti-inflammatory Agents
- Acetylsalicylic acid (A)[a]
- Ibuprofen (Motrin) (A)
- Indomethacin (Indocin) (A)
- Phenylbutazone (Butazolidin) (A) (T)[b]
- Tolmetin (Tolectin) (A)

Antimicrobials
- Amoxicillin (A) (T)
- Cefazolin (Ancef) (A) (T)
- Cephalothin (Keflin) (A) (T)
- Cephradine (Velosef) (A)
- Chloramphenicol (A) (T)
- Erythromycin (T)
- Isoniazid (A) (T)
- Metranidazole (Flagyl) (A)
- Oxacillin/Methicillin (A)
- Penicillin (T)
- Pentamidine (T)
- Tetracycline (T)
- Vancomycin (A)
- Vidarabine (A) (T)

Miscellaneous Agents
- Chlorthiazide (Diuril) (T)
- Cimetidine (Tagamet) (A) (T)
- Diazepam (Valium) (T)
- Diphenylhydantoin sodim (Dilantin) (A) (T)
- Heparin (T)
- Phenothiazines (Thorazine, Compazine) (A) (T)
- Sulfisoxazole (Gantrisin) (A) (T)
- Tolbutamide (Orinase) (T)
- Trimethoprim–sulfamethoxazole (Bactrim) (A)

[a] (A): implicated in Agranulocytosis.
[b] (T): implicated in thrombocytopenia.

kocytosis,[79,83] eosinopenia,[83] lymphocytopenia,[102] and monocytopenia.[102] The neutrophilic leukocytosis results from decreased intravascular efflux and marrow release.[19,25] Dexamethasone may differ from prednisone by enhancing local leukocyte migration.[252] These agents affect neutrophil function by decreasing hexose monophosphate shunt activity,[216,254] decreasing the bactericidal activity against *E. coli,*[216,254] and inhibiting neutrophil chemotaxis,[29,252] granulocyte adherence,[211] and phagocytosis.[138] They suppress nitroblue tetrazolium reduction,[61] presumably by inhibition of NADH oxidase. The monocyte–macrophage system is inhibited through decreased monocyte–macrophage clearance[334] as well as decreased bactericidal and fungicidal activity of macrophages as demonstrated *in vitro.*[271] The net result of these diverse effects is increase the susceptibility to infectious complications of patients receiving corticosteroids. The intermittent nature of glucocorticoid administration allows the reconstitution of neutrophil function during treatment-free periods. However, the cytotoxic effects of chemotherapy resulting in neutropenia allows little time for patients to completely reconstitute leukocyte number and function for adequate infection-fighting capability.

Hematologic and Antibiotic Support

Frequent blood transfusions are rarely needed during chemotherapy in the absence of infection or bleeding. They are used most often in the treatment of patients with hematopoietic or solid tumors receiving combined chemotherapy and radiotherapy, during which the ability of the compromised marrow to respond to stress is decreased for prolonged periods of time. Patients tolerate hemoglobin levels greater than 8 g/dl reasonably well, and in the absence of cardiovascular or severe pulmonary disease a conservative approach to blood transfusions should be taken. If necessary, only packed red blood cells should be given. Hepatitis may result from multiple blood transfusions. Rarely, bone marrow aplasia ensues after such an infection[135,292] and is nearly always fatal.

Animal studies[78,98] and several human studies[9,14,146,149,284] have demonstrated the utility of granulocyte transfusions in improving survival and response to infection in the granulocytopenic host. At the present time this product should only be used when the neutropenic state (less than 500×10^9/l) is expected to last for longer than 3–4 days and the patient has a documented infection not responding within the first 24–48 hours to broad-spectrum antibiotic coverage. Although several studies have appeared to demonstrate some benefit from prophylactic granulocytes,[66,110] the indications for this are still unclear. The use of random granulocyte transfusions is to be discouraged, and the best HLA-matched granulocytes should be used, since the response to infection appears to be less than optimal in previously sensitized animals.[332] Granulocytes in adequate quantities can now be harvested by a number of instruments and techniques[88,127,150] available in most medical centers. Platelet support is discussed below.

Glucocorticosteroids,[318] androgens,[176] growth hormone,[124] and lithium[277,305] have been reported to enhance hematopoiesis in various culture systems and occasionally *in vivo.* Pharmacologic stimulation of bone marrow function in hypoplastic states induced by chemotherapy may become possible once the complex interaction between these agents and stem cell production, maturation, and cell release is clarified. The treatment of qualitative disorders induced by antineoplastic drugs is difficult in view of the necessity to continue their administration to treat both the malignancy and complications. Replacements of individual blood products is necessary, and even these may not function effectively until the tumor and its complications are controlled and therapy is withdrawn.

The quantitative and qualitative defects induced in lymphoid tissue and the monocyte–macrophage system by chemotherapeutic agents[206] severely impair the defense against infection. The recovery of immune competence depends upon the duration of administration of chemotherapy agents and control of the underlying disease; it should be remembered, however, that a depressed immune response is not uncommon in patients with malignancy, independent of treatment. Defects in immune function may be seen long after therapy is stopped.[33]

Antimicrobial prophylaxis utilizing trimethaprim–sulfamethoxazole[97] to prevent both bacterial and *Pneumocystis carinii* infections[158] is of benefit. Patients respond to vaccination against viruses by antibody production, although with a higher failure rate in eliciting "protective" antibody titers.[286] It is important to keep in mind that almost any organism whether considered a classic pathogen or not, may be responsible, for infection in the

immunosuppressed host, and broad-spectrum antibiotics, antifungal agents, and antiprotozoic agents may have to be instituted, often concurrently.

Hemostatic Abnormalities

Normal hemostasis depends upon a complex balance between blood vessel integrity, platelet function, and blood clotting factor interactions. In patients with lymphoma each of these aspects may become abnormal, with resultant hemorrhage or thrombosis, depending upon whether the balance of elements is inhibited or stimulated (Table 10–5).

Blood Vessel Wall Abnormalities

It is obvious that normal anatomic continuity must be maintained in order for blood to remain within the vascular space. Although reflex vasoconstriction occurs, it is unlikely to represent a significant hemostatic response, at least in humans.[147] The secretory function of endothelial cells results in products that play a significant role in the promotion of platelet adhesion,[162] inhibition of platelet aggregation (prostacyclin secretion)[333] and thrombus dissolution through plasminogen activator release and subsequent fibrinolysis.[319] The laboratory diagnosis of a vascular purpura is based upon a prolonged bleeding time in a patient with a normal number of platelets and normal platelet functions *in vitro.*

TABLE 10–5 Disorders of Hemostasis in Patients with Lymphomas

- I. Blood vessel wall abnormalities
  - A. Endothelial injury (immune complexes, infection)
  - B. Amyloid deposition
- II. Platelet abnormalities
  - A. Thrombocytopenia
    - 1. Decreased production
      - a. Treatment related
      - b. Nutrient deficiency
      - c. Myelopthisis
      - d. Drug induced
      - e. Infections
    - 2. Peripheral destruction
      - a. Immune mediated
      - b. Infection related
      - c. Hypersplenism
      - d. Intravascular coagulation syndromes
  - B. Thrombocytopathy
    - 1. Drug induced
    - 2. Lymphoma related
    - 3. Antibody mediated
    - 4. Uremia
    - 5. Fibrin split products
- III. Coagulation abnormalities
  - A. Liver disease related
  - B. Coagulation factor antibodies/inhibitors
  - C. Intravascular coagulation syndromes
    - 1. Disseminated intravascular coagulation
    - 2. Deep-vein thrombosis and pulmonary embolus
    - 3. Central nervous system thrombosis

Purpuric lesions caused by vascular disorders are uncommon when compared to those caused by thrombocytopenia. Kaposi's sarcoma, now seen with increasing frequency among the homosexual population,[160] represents a neoplasm of vascular tissue[233] and is not strictly a lymphoproliferative disorder or purpura, but must be considered in the differential diagnosis of lymphadenopathy in young males because of the ability of this tumor to proliferate within nodal sites. Immune complexes,[179] as well as viral[47] or bacterial[223] infections, may result in vascular injury. Infectious purpuras are partly mediated through endothelial injury induced by endotoxin.[223] These forms of purpura are rarely observed unless associated with thrombocytopenia or disseminated intravascular coagulation. Corticosteroid administration over prolonged periods results in easy bruisability, thought to be a result of alterations of dermal–vascular support and decreased platelet adhesion.[55] Short-term administration of steroids may improve vascular integrity—an observation thus far demonstrated only *in vitro*[210] or *in vivo* in animal models.[191] "Purpura cachexia" is observed in states of negative nitrogen balance and is thought to result from a decreased production of the supporting tissue matrix surrounding the endothelial cells.

Plasma cell dyscrasias are often associated with purpura. Amyloid deposits may infiltrate within vascular walls, causing enhanced fragility[15] and subsequent hemorrhage. In both Waldenström's macroglobulinemia and multiple myeloma, purpura is common.[249,250] Although the chronic administration of corticosteroids and sludging of blood with subsequent hypoxia and tissue injury are partly responsible for vascular fragility, thrombocytopenia secondary to disease and therapy, an acquired thrombocytopathy,[250] and circulating

anticoagulants[250] are more commonly implicated. Types I and II cryoglobulinemia associated with lymphoproliferative disorders[42,133] frequently presents with lower extremity purpura.[42,133] The treatment of a vascular purpura depends upon its etiology. Immune-complex purpura and purpuras associated with dysproteinemias are treated with plasmapheresis, immunosuppressive agents, and antineoplastic combination chemotherapy. Infectious purpuras resolve with appropriate antimicrobial therapy. Amyloid deposition is a particularly difficult problem to treat. Reports of the efficacy of dimethylsulfoxide[246] need to be confirmed.

Platelet Abnormalities

Platelets play a central role in normal hemostasis by adhering to areas of vascular injury, aggregating to close minute breaches in the vascular endothelium, and functioning as a solid-state system on which the coagulation factors interact to form a fibrin mesh. Both decreased platelets and poorly functioning platelets may cause severe hemostatic abnormalities.

Thrombocytopenia is one of the most common abnormalities observed in patients with lymphoproliferative disorders. Decreased production is most often related to chemotherapy or radiotherapy and is commonly the limiting factor, along with neutropenia, in the administration of these modalities of therapy. In addition to this direct effect by inhibition of megakaryocyte production, ineffective thrombocytopoiesis may be present in megaloblastic anemias[140]—disorders in which platelet function may be abnormal as well.[200] Iron appears to have a regulatory role in thrombopoiesis.[173] Thrombocytosis,[288] thrombocytopenia,[207] and normal platelet counts[60] have been reported in clinical and experimental iron deficiency. Lymphomatous involvement of the marrow may impair thrombopoiesis, resulting in thrombocytopenia.[140] Corticosteroids have been implicated in a selective depression of thrombopoiesis,[70] possibly as a direct result of their catabolic properties. A number of drugs, apart from chemotherapy agents, used in patients with lymphoproliferative disorders have been associated with neutropenia and thrombocytopenia (Table 10–4). Infections, particularly those of a viral nature, may inhibit thrombopoiesis through damage to megakaryocytes. Morphologic abnormalities in megakaryocytes, accompanied by thrombocyopenia, have been observed in cytomegalovirus inclusion disease,[57,107,141,243] varicella zoster,[99] and rubella.[245] A particularly severe form of marrow hypoplasia–aplasia may occur following hepatitis B infection.[136]

Peripheral destruction of platelets is an important cause of thrombocytopenia in patients with lymphoma. This may be due to an immune-mediated reaction (either drug induced or "idiopathic"), a direct effect of infectious agents on platelets with subsequent activation and increased clearance, hypersplenism, or intravascular coagulation syndromes. Immune-mediated thrombocytopenia is not uncommon in patients with lymphoproliferative disorders.[89] This has been described in CLL,[30,51,90,167,170] Hodgkin's disease,[69,142,167,169,170,280,281,327] and other non-Hodgkin's lymphomas.[105,167,170] The pathophysiology of platelet destruction is similar to that of red blood cell destruction in autoimmune hemolytic anemia, with binding of the IgG antibody to the platelet associated antigen and subsequent macrophage clearance.[213] Immune thrombocytopenia may herald the appearance of the lymphoma, occur simultaneously with the diagnosis, be a harbinger of relapse, or present after apparent cure of the disease. Drug-induced immune thrombocytopenia must be considered in any patient with unexplained thrombocytopenia receiving medications. Drugs proven to have caused immune mediated destruction include hydrochlorthiazide,[93] diphenylhydantoin,[63] diazepam,[63] and sulfisoxazole.[63] A severe form of infection-related immune thrombocytopenia has been described in Epstein-Barr virus infection.[95] Clinically, these disorders (both "idiopathic" and drug induced) present with thrombocytopenia, a marrow containing normal to increased numbers of megakaryocytes, and absence of splenomegaly or abnormal coagulation studies.

The treatment of autoimmune thrombocytopenia depends upon the clinical status of the patient. If no lymphoma is present at the time of the thrombocytopenia, corticosteroids are considered the mainstay of treatment. Prednisone is administered at a dosage of 1 mg/kg/day until a maximum platelet count is achieved and then is tapered slowly.[213] A splenectomy is indicated if there is no response to steroids, if tapering results in a relapse, if complications secondary to corticosteroids become unmanageable, or if borderline splenomegaly is present, suggesting the possibility of recurrent lymphoma. Cytotoxic agents may

have to be used; these include vinca alkaloids[6,218] and bleomycin.[253] In the presence of active lymphoma, the occurrence of thrombocytopenia must be placed in the perspective of the total clinical picture. In this setting, treatment of the underlying lymphoma is most appropriate; this is generally successful in reversing the thrombocytopenia, since most lymphoma regimens contain a corticosteroid and a cytotoxic agent. Heparin-associated thrombocytopenia, due to an antigen–antibody reaction[321] or complement-mediated platelet injury,[64] has been reported and may be related to the type of heparin administered, particularly the bovine lung preparation.[23] Once a drug-induced thrombocytopenia is suspected, the administration of the agent must be discontinued.

The etiology of infection-related thrombocytopenia is complex.[242] Bone marrow suppression, as noted above, is only a partial explanation. Immune destruction may occur through antigen–antibody complexes causing platelet aggregation.[232] Viral infections induce changes in platelet surface membranes and increase splenic clearance with subsequent thrombocytopenia,[293] as well as directly cause platelet aggregation.[293] Bacterial infections result in platelet destruction through injury to vascular endothelium[223] and subsequent adhesion, endotoxin damage to endothelial cells and platelets,[156] direct bacterial–platelet interactions,[65] macrophage clearance of antibody–platelet complexes,[175] and intravascular coagulation.[12]

Splenic sequestration is an important cause of thrombocytopenia (see above). It has been estimated that almost 90% of the total circulating platelet mass may be sequestered in an enlarged spleen.[18] In view of the frequent occurrence of splenomegaly in the lymphoproliferative disorders, it is not surprising that discussions as to the therapeutic benefit of splenectomy are quite common. As noted above, hairy cell leukemia and primary splenic lymphoma are the only clinical situations in which splenectomy is the primary treatment of choice. In most other lymphoproliferative disorders, chemotherapy is successful in decreasing splenic size. It is generally in those patients with persistant splenomegaly who have become resistant to therapy that platelet sequestration becomes a clinical problem. In CLL, splenomegaly may be resistant to chemotherapy and splenectomy may be successful in improving hematologic parameters significantly, even in patients considered far advanced by staging criteria.[279] Although radionuclide studies can successfully predict that platelet sequestration is significant, thrombokinetic studies are not readily available to predict "normal" marrow responses, and therefore a splenectomy, even in the face of apparently increased sequestration, does not always result in a "normalization" of platelet count. Splenic irradiation has been used in these disorders[49,87] with occasional success and must be considered if surgical intervention is contraindicated. Occasionally, elevated platelet counts have been observed in malignancy[20,198] and may be partly responsible for the increased incidence of thromboembolism (see below). This is rare in lymphoproliferative disorders in the nonsplenectomized individual.[172]

In addition to a normal number of platelets, their proper function is important. There are a number of qualitative disorders of platelet function (thrombocytopathy) in patients with lymphoproliferative diseases (Table 10–5), and although bleeding is more commonly associated with thrombocytopenia, these abnormalities must be taken into consideration when one is evaluating a patient because of bleeding. Aspirin and aspirin-containing compounds are the most common agents inducing a thrombocytopathy.[100,276] Other drugs that are used in this patient population and have been shown to inhibit platelet function include indomethacin,[266] carbenicillin,[43] ticarcillin,[44] other penicillin compounds,[45] and various phenothiazines.[163] A number of chemotherapeutic agents have been reported to affect platelet function *in vitro* and occasionally *in vivo;* these include melphalan,[178] cyclophosphamide,[148] actinomycin D,[148] vincristine,[306] and daunorubicin.[257] Mithramycin causes a prolonged bleeding time, decreased platelet aggregation induced by adenosine diphosphate, collagen, and ephinephrine, and decreased platelet adenosine diphosphate.[7]

Complications secondary to the presence of lymphomas may result in abnormalities of platelet function significant enough to cause bleeding. Platelet function is intrinsically abnormal in a number of myeloproliferative states.[118] In hairy cell leukemia a platelet abnormality has been defined with subsequent improvement after splenectomy reported. The latter finding suggests that there is not an intrinsic platelet abnormality (as in the myeloproliferative syndromes), but a nonspecific effect of diseased splenic tissue. There is no evidence at this time that platelet abnormalities result from the hypersplenic state. Paraproteins

may interfere both with platelet function[250] and coagulation (see below). The treatment of the underlying dysproteinemic state is adequate. Occasionally plasmapheresis may be necessary[283] to control these abnormalities.

Von Willebrand's factor is essential for normal platelet function. Several case reports have appeared describing antibodies against this factor and subsequent decreased platelet function in patients with lymphoproliferative diseases.[166,214,330] Uremia may result from a rapidly progressive, obstructing abdominal lymphoma; or because of an intrinsic kidney abnormality, as in myeloma. Platelet function is significantly impaired in the uremic state, and a number of abnormalities have been described.[263] The hemostatic defect is corrected by destroying the obstructing tumor mass if possible, by dialysis,[263] or, with the use of cyryoprecipitate.[164] Fibrin and fibrinogen degradation products, formed during intravascular thrombosis, may not be cleared adequately if liver disease is present[267] or a significant degree of monocyte–macrophage function is absent (i.e., splenectomy).[296] These products interfere in both platelet function[185] and coagulation (see below). Correction of these abnormalities depends upon both the prevention and treatment of thromboembolic disease.

Coagulation Abnormalities

In normal hemostasis, a series of plasma proteins interacts in such a fashion as to result in a fibrin mesh and subsequent closure of vascular injury. Because of the complex interaction of these proteins with vascular endothelium, circulating nonclottable proteins, and circulating hematopoietic cells, both benign and malignant, it is not surprising that abnormalities and subsequent hemorrhage and/or thrombosis are frequent in patients with lymphoma. The contact activation system (the intrinsic pathway) depends upon the activation of factor XII in the presence of factor XI, plasma prekallikrein, and high molecular weight kininogen.[224,299] Once this initial step occurs, further coagulation factors interact, and fibrin is eventually generated through a common pathway involving factors X and V, prothrombin, and fibrinogen. Congenital factor deficiencies of this pathway are common, particularly factor XI deficiency and von Willebrand's disease, and must be considered in the differential diagnosis of abnormal coagulation studies in lymphoma patients. Tissue thromboplastin interacts with factor VII (extrinsic pathway), and subsequently the common pathway is activated and fibrin formation results. Both pathways may be abnormal in lymphoma patients secondary to acquired abnormalities.

Because most coagulation factors are produced in the liver, abnormalities of liver function (induced either by a drug or by tumor invasion) will result in both quantitative and qualitative abnormalities. Vitamin K is a necessary cofactory in the biosynthetic pathway of factors II, VII, XI, and X in the liver. With the exception of biliary obstruction and other malabsorption syndromes, deficiencies of vitamin K result from a combination of deficient intake and gut sterilization by chronic antibiotic administration.[17] Just such a situation may arise in hospitalized patients undergoing intensive therapy. In the presence of liver disease, the biosynthesis of non–vitamin K dependent coagulation factors is impaired.[267] Liver disease may also cause the production of abnormal clotting factors,[195,214] as well as a vitamin K–dependent decarboxylation deficiency.[27] In addition to deficient and abnormal synthesis, other abnormalities induced by liver disease, particularly cirrhosis, include defective clearance of activated clotting and fibrinolytic enzymes and fibrin split products,[267,296] accelerated breakdown of fibrinogen–fibrin,[108] defective polymerization of fibrin,[10] disseminated intravascular coagulation,[85] and hypersplenism.

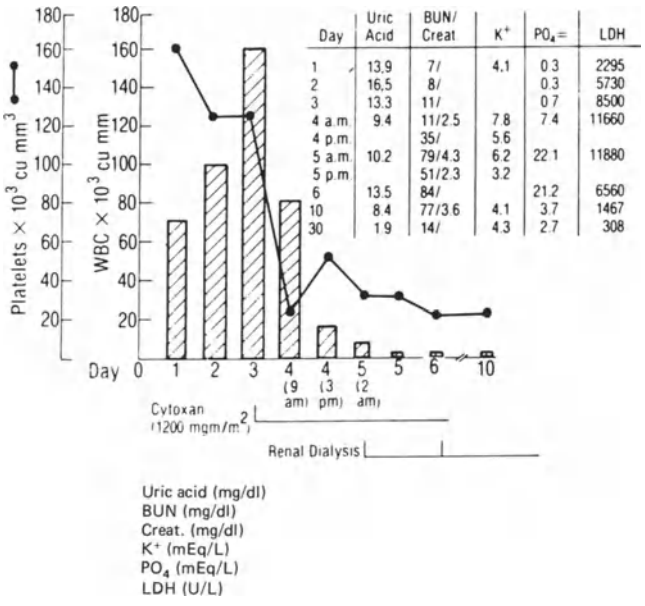
Although antibodies directed specifically against each coagulation factor have been described, they are most often seen in patients with congenital deficiencies and only rarely in patients with lymphoma. Antibodies to factor VIII are the most commonly encountered as fluid-phase anticoagulants, occasionally related to penicillin administration.[128] A paraprotein has been described as having specific anticoagulant activity against factor VIII.[121] A patient with Waldenström's macroglobulinemia immunoabsorbed factor VIII onto lymphoma cells; the latter disappearing after splenectomy.[41] Occasional patients taking chlorpromazine[120] or diphenylhydantoin[258] have developed such an antibody. An acquired von Willebrands' disease due to an antibody has been described in several patients with lymphoma;[166,217,330] the antibody is directed against the antigenic determinant on the factor VIII molecule. Treatment of the underlying lym-

phoma resulted in the disappearance of the antibody. An inhibitor, described in systemic lupus erythematosis and recognized as either an IgG or IgM, has been observed in a number of conditions, including lymphomas.[37,287,317] The anticoagulant is directed against the enzyme complex of "prothrombinase" (Xa-V-phospholipid), inhibiting the conversion of prothrombin to thrombin in the common pathway.[40] Although relatively commonly encountered during the laboratory evaluation of patients with malignancy, clinically significant bleeding is rare[287] and thromboembolic phenomena have been described.[229] A paraprotein with this specificity has been reported.[317] An IgA paraprotein demonstrating antithrombin activity has also been described.[309] Both primary and secondary amyloidosis may be associated with decreased levels of factor X.[24,116,157,184,248] The pathophysiology of this deficiency appears to be an adsorption of factor X on amyloid material.[115] This hemostatic abnormality in amyloidosis is compounded by associated factor deficiencies,[262] accelerated fibrinolysis,[251] intravascular coagulation,[262] decreased vascular integrity,[15] and platelet dysfunction subsequent to uremia.[263] Amyloid deposition in the kidney may result in the nephrotic syndrome. Both albumin and numerous coagulation factors (including antithrombin[196]) may be lost.[155] The loss of antithrombin may result in a hypercoagulable state and subsequent thrombosis.[151] Several reports of coagulation abnormalities in lymphoma patients have been published and a variety of abnormalities, particularly those related to the fibrinolytic enzyme system, have been described.[118,119,220,240]

Fibrin deposition within the vascular space results in a spectrum of disorders, collectively known as "hypercoagulable states." These include disseminated intravascular coagulation, deep vein thrombosis, pulmonary emboli, sagittal sinus thrombosis, and possibly nonbacterial thrombotic endocarditis. Normally, circulating plasma proteins (antithrombin III, α_2-macroglobulin) inhibit this deposition, and the fibrinolytic enzyme system as well as the leukofibrinolytic capacity of neutrophils and macrophages dissolve intravascular thrombi and permit the resumption of normal blood flow. Hypercoagulability results either when these control mechanisms are absent (e.g., decreased levels of protein C in intravascular coagulation[132] or antithrombin III in the nephrotic syndrome of amyloidosis[196]) or when the amount of fibrin formed is so great as to overwhelm the normal protective mechanisms (e.g., a tumor lysis syndrome). Depending upon the degree of deposition and certain anatomic considerations, a variety of syndromes may occur.

Disseminated intravascular coagulation (DIC) is the most generalized syndrome. Fibrin is deposited throughout the vasculature of the body with subsequent utilization and depletion of coagulation factors and platelets. This results in bleeding, and a paradoxical state in which hemorrhage and thromboses are occurring simultaneously. Both the intrinsic and extrinsic coagulation pathways may be stimulated to produce thrombin through the common pathway (X $\rightarrow$ Xa), the former through the activation of Hageman factor during sepsis[219] and the latter by release of procoagulant material from neoplastic tissues. Thrombin is subsequently responsible for fibrin generation from fibrinogen. The release of thromboplastic material from lymphoma cells after therapy of a lymphoblastic lymphoma is illustrated in Figure 10–2. Fulminant DIC resulted, which was treated with heparin, clotting factors (plasma), and platelets; it subsided once maximum tumor cell kill occurred. DIC is unusual in those lymphoproliferative disorders in which cell proliferation is minimal (CLL, nodular lymphoma, multiple myeloma, and Waldenström's macroglobulinemia), but is possible in other disorders in which the cells may be exquisitely sensitive to therapy and rapid cell lysis results (lymphoblastic lymphoma, acute lymphoblastic leukemia, diffuse lymphomas).

Septicemia, a common problem in lymphoproliferative disorders, is a major cause of abnormalities of blood coagulation and DIC in all series published.[12,76,298] A particularly severe form may be observed in the setting of pneumococcal sepsis in splenectomized patients,[26] secondary to a defect in adequate clearance of both the organisms and activated clotting factors. Endotoxin has numerous effects upon the hemostatic mechanism, including activation of Hageman factor,[219] release of tissue thromboplastin from leukocytes[237,238] and monocytes[324] and subsequent activation of the common pathway, and endothelial injury,[223] resulting in platelet adhesion, aggregation, and intravascular thrombosis. Endotoxin has weak platelet aggregant activity against human platelets.[156,268] Gram-positive organisms that lack endotoxin may trigger DIC by tissue injury and activation of the contact-activation system (factor XII).[324] Immune


Platelets × 10³ cu mm
WBC × 10³ cu mm
Day 0 1 2 3 4 (9 am) 4 (3 pm) 5 (2 am) 5 6 10
Cytoxan (1200 mgm/m²)
Renal Dialysis
Uric acid (mg/dl)
BUN (mg/dl)
Creat. (mg/dl)
K⁺ (mEq/L)
PO₄ (mEq/L)
LDH (U/L)

Day	Uric Acid	BUN/ Creat	K⁺	PO_4=	LDH
1	13.9	7/	4.1	0.3	2295
2	16.5	8/		0.3	5730
3	13.3	11/		0.7	8500
4 a.m.	9.4	11/2.5	7.8	7.4	11660
4 p.m.		35/	5.6		
5 a.m.	10.2	79/4.3	6.2	22.1	11880
5 p.m.		51/2.3	3.2		
6	13.5	84/		21.2	6560
10	8.4	77/3.6	4.1	3.7	1467
30	1.9	14/	4.3	2.7	308

FIGURE 10–2. Disseminated intravascular coagulation occurring during a tumor lysis syndrome. This 39-year-old woman was admitted with the diagnosis of diffuse, poorly differentiated lymphoma, stage IV. One dose of cyclophosphamide (1900 mg i.v.) was given on day 3. Shortly thereafter, massive tumor lysis occurred with a syndrome characterized by DIC, metabolic acidosis, renal failure, hyperphosphatemia, and hyperkalemia. Heparin, allopurinol, sodium bicarbonate, kayexalate, insulin, and eventually renal dialysis were used successfully and the patient achieved a remission.

complexes are capable of activating factor XII[219] as well as causing platelet aggregation; if present, they may be considered a contributing factor to thrombosis in patients with lymphoproliferative disorders.

The treatment of DIC depends both on the degree of activation of the coagulation mechanism and the etiology. Although more commonly seen in carcinomas, subclinical DIC may occur in patients treated for lymphoproliferative disorders. Treatment is directed towards the underlying disorder. However, in fulminant DIC, seen after treatment of a rapidly proliferating lymphoma, heparin therapy may be lifesaving. Obviously, if infection is present this must be treated as well. Pneumococcal sepsis in the splenectomized individual often demands intensive care with cardiovascular monitoring, antibiotic therapy, and plasma and platelet replacement as well as short-term heparin therapy. This problem is less frequent with the institution of presplenectomy pneumococcal immunization and postsplenectomy antibiotic prophylaxis.

Deep-vein thrombosis and pulmonary emboli[126] are significant problems in patients with cancer and result in additional morbidity. In patients with lymphoma, the cause of deep-vein thrombosis is most frequently venous obstruction from enlarged inguinal and retroperitoneal lymph nodes. The incidence of this complication is not known, but it is a frequent clinical observation. Heparin therapy and treatment of the local obstruction with radiotherapy are necessary since either alone may not result in a satisfactory clot resolution. Subcutaneous heparin may prevent such complications in the bed-ridden patient.[116] Sagittal sinus thrombosis is being recognized as a complication of induction therapy in acute lymphoblastic leukemia.[307] Although seen in the absence of the administration of L-asparaginase, this agent is frequently associated with this complication. The mechanism of this hypercoagulable state is thought to be a decrease in antithrombin III induced by L-asparaginase.[260] Other coagulation abnormalities induced by this agent include hypofibrinogemia and decreased factor VIII.[265] It is unclear why the cerebral vessels are particularly susceptible if this general mechanism of protection against thrombosis is absent. Retinal vein thrombosis was described in a patient with multiple myeloma and a cryoglobulin.[335] Nonbacterial thrombotic endocarditis is present in a significant percentage of cancer patients, mainly in the terminal state, but it is difficult to diagnose except at autopsy. Its incidence in patients with lymphoma is not known. The diagnosis is based upon the clinical presentation of multiple neurologic signs and symptoms, the presence of thrombi in the cerebral circulation, and premortem demonstration of valvular vegetations containing fibrin and platelet thrombi. Although this problem has significant importance in the differential diagnosis of neurologic

syndromes[74] in cancer patients, the poor prognosis of patients with this entity generally mitigates against aggressive treatment approaches.

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CHAPTER 11

Cell Kinetics and the Classification of Lymphocytic Lymphomas

Herbert Hansen

The wide spectrum of neoplasms of the lymphatic system mirrors the functional heterogeneity of its cellular elements. As discussed in other chapters in this volume, patients with disseminated forms of these diseases may live with them for many years under only partial control (as in the case of nodular lymphomas) or may succumb within a period of months, even with the best available treatments (as in Burkitt's lymphoma).

There are many possible explanations for these variations in natural history and response to therapy. Among those features proven in the past to influence survival in large groups of patients are the quantity of neoplastic cells in the host at the time of diagnosis (tumor cell burden),[18,66] location of disease in various body compartments (extent of disease),[18,30,37], histopathologic appearance of involved tissues and cytologic appearance of neoplastic cells,[19,37,44], and host factors (e.g., age, previous treatment).[30,34]

Recently, additional biologic features of the neoplastic cells have come under study and promise to give new and important powers to the physician in the prognostication of the diseases and the design of more specific therapies. One area of study is the proliferation and differentiation of neoplastic cells, comparing these features with those of normal cells both in the ontogeny of the lymphatic system and during the development of cellular immune responses.[4,5,62] Another is the development of improved methods for the growth and study of neoplastic cells *in vitro*.[32,46] Still other research has resulted in newer concepts of cellular "resistance" to the cytotoxic effects of antineoplastic agents and a more detailed understanding of the patterns of overall cellular proliferation in many types of lymphatic neoplasms.[8,22,28,45,49,56] The present chapter will address primarily the latter research and attempt to show how it can be applied to the classification and therapy of some of these disorders.

Cell Kinetic Methods

It is not the purpose of this chapter to detail the complex theory and methodology involved in the study of the growth kinetics of tumor cells, which has been aptly described in many monographs and reviews,[21,61] however a brief outline may be helpful to the reader unfamiliar with these techniques.

In Vivo Studies of Human Cellular Proliferation

The most detailed information presently available describing the growth of human neoplastic cells *in vivo* has come from studies using parenteral injections or infusions of nucleosides tagged with radioactive tracers. In most studies the pyrimidine nucleoside thymidine, tagged with either a tritium ion or carbon-14, has been employed. This nucleoside, after phosphorylation, is incorporated with a high degree of specificity into newly synthesized molecules of DNA. Cells that are not in the DNA-synthetic portion of the cell growth cycle (S-phase) during the period of exposure incorporate little, if any of the radioactivity into their cellular material, and hence the tracer serves as a marker for a specific cell-cycle–related event, i.e., DNA synthesis. Cells that have incorporated the tracer into

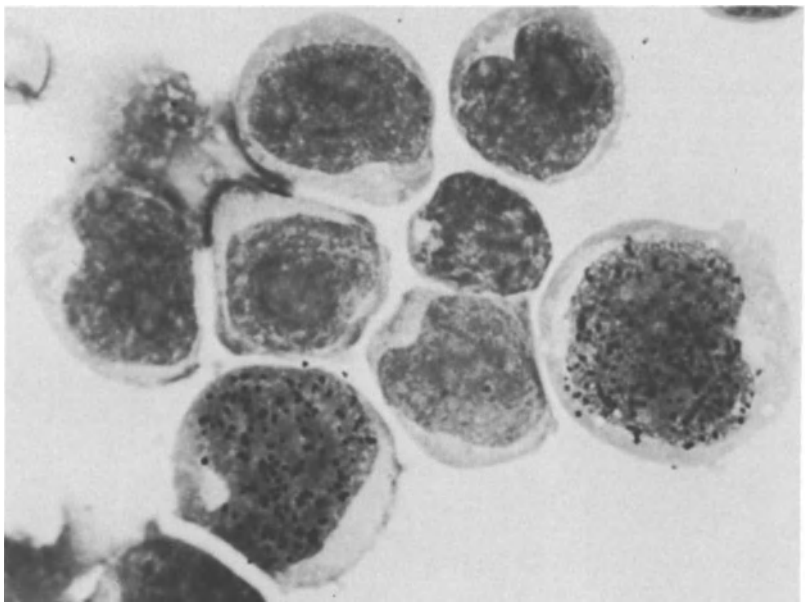

FIGURE 11–1 ^{3}H-Thymidine autoradiography. Autoradiograph of cells from a lymph node biopsy suspension of a patient with diffuse histiocytic lymphoma (Rappaport classification). Black grains of reduced silver appear over the nuclei of cells that were in the S-phase during the period of *in vitro* exposure to ^{3}H-thymidine. The percentage of labeled cells in the population is the "labeling index" for the tumor specimen. (680×)

their DNA are identified by removing them from the involved tissues in thin microtome sections or single-cell suspensions, placing them on glass slides, and overlaying them with a photographic emulsion or film. After exposure of the film for various periods of time, the slides are developed by standard photographic methods, stained, and examined by light microscopy. Cells that have incorporated the radioactive thymidine show black grains over the nucleus where beta particles emitted from the tracer have reduced ionic silver in the film or emulsion (Figure 11–1). These cells are considered to have been "labeled" and the fraction of such cells in the entire population is termed the "labeling index" (LI).

By serial examinations of the cell population under study after a single bolus injection of radioactive thymidine, or during and after a continuous infusion, the fate of the tracer in proliferating cells can be determined, and various parameters of overall growth can be estimated for the population as a whole. Some estimates that can be obtained from this type of study are the following:

1. Mean population cell cycle time: average time taken by proliferating cells in the population to pass through a single generation cycle.
2. S-phase duration: duration of DNA synthetic period (S-phase).
3. Postsynthetic, premitotic interval: duration of interval between completion of DNA synthesis in preparation for mitotic cell division and the onset of the mitotic phase of the cell cycle.
4. Mitotic interval: duration of the various phases of mitosis, ending in the production of two daughter cells.
5. Growth fraction: fraction of cells committed to the mitotic division cycle at any given time.
6. Cell loss rate: fraction of cells lost from the population during any given interval of study.

In Vitro Studies of Human Cell Proliferation: The "Flash" Thymidine Labeling Index

Cells freshly obtained from tumors or body fluids are placed in appropriate tissue culture medium in the presence of ^{3}H-thymidine. A short (1–2 hour) incubation time is used with or without an enhanced oxygen atmosphere. The cells are then washed free of the external thymidine, fixed, and analyzed by the autoradiographic methods described above. If the cells are exposed immediately, or within a short time of their removal from the tissue, the resulting LI approximates closely the value obtained by short-exposure *in vivo* labeling.[41,57]

The fraction of cells in S-phase may also be measured by newer methods of automated cytology. The technique of flow cytometry, detailed elsewhere,[21] utilizes molecular stains for DNA, which, when excited by an intense light source

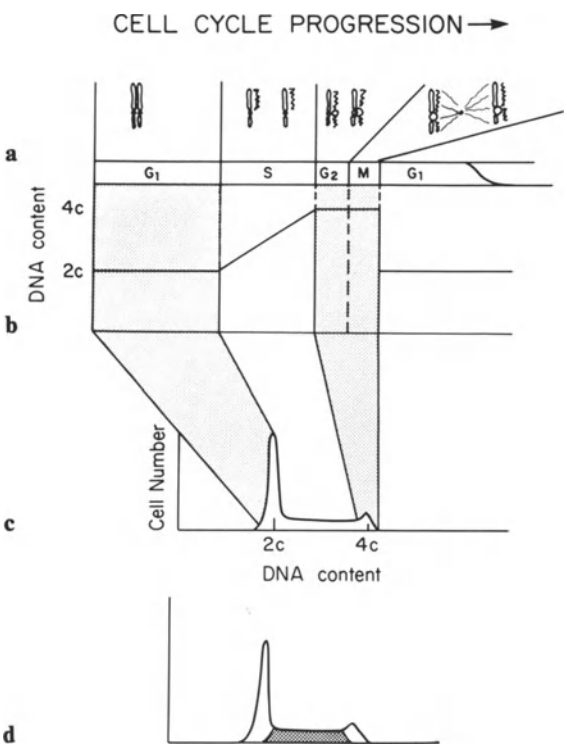

FIGURE 11–2 Flow cytometric cell cycle analysis. **(a)** Chromosome duplication during the mitotic cycle, entailing progressive increase in cellular DNA content as the cell progresses through the phases of the generative cycle: G_1, period prior to DNA synthesis; S, DNA synthetic period; G_2, postsynthetic, premitotic period; M, mitosis **(b)** Changes in DNA content during various cell cycle phases: 2c, DNA content corresponding to the normal diploid chromosome complement; 4c, DNA content corresponding to a state in which chromosomes have duplicated themselves. **(c)** DNA histogram of a proliferating cell population. **(d)** Deconvolution of the histogram allows calculation of the fraction of cells in the DNA-synthetic portion of the cell cycle (S-phase fraction), shown as the shaded area under the curve.

such as a laser or mercury vapor lamp, emit fluorescent light of an intensity proportional to the amount of DNA present in a cell. Thus the content of DNA in large numbers of individual cells can be measured and compared to the DNA content of normal diploid control cells. Since the DNA content of cells increases as they pass through the cell cycle (Figure 11–2a,b), S-phase cells can be identified by this measurement, and their relative numbers in the population calculated (Figure 11–2c,d). There is good agreement between S-phase fractions measured by this technique and by the LI method.[3]

Proliferation of Cells of the Normal Immune System

Cell proliferation is a central element of the function of the normal immune system. The requirement to respond to a vast array of possible threats to the integrity of the organism is met by systems allowing amplification of functional cell subsets to meet specific challenges. Amplification is obtained through controlled cell proliferation in lymphoid tissues.

Detailed cytokinetic studies of lymphoid cell populations *in vivo* are hampered by the extreme complexity of their tissues. Composed of both permanent and transient cell populations, with a wide spectrum of functional, regulatory, and proliferative behaviors, these tissues are difficult to analyze by classical cell kinetic techniques. For this reason, among others, much of what we know about the proliferation of lymphoid cells has been gained from the study of *in vitro* models of immune responses, where the cell populations under study can be better defined.

Lymphocyte Proliferation in Response to Mitogens

The small lymphocyte of peripheral blood is not an end-cell; rather, it is a cell partially differentiated to membership in the immune system, in a kinetically quiescent intermediate state, retaining limited capacity for further proliferation and functional differentiation. When stimulated *in vitro* by external mitogenic molecules, these cells undergo a self-limited series of proliferation cycles, intimately coupled with further differentiation to end-stage cells with specific immune functions. There is considerable evidence that parallel events occur *in vivo* in response to immune stimuli.

The discovery by Nowell, reported in 1960,[47] that kinetically quiescent blood lymphocytes could be stimulated to proliferation by the plant lectin phytohemmagglutinen (PHA) began an intensive study of the biology of the immune response *in vitro*. Later studies showed that this proliferation was not a response to the antigenicity of the PHA molecule, but was the result of a nonspecific stimulus to a large subset of lymphocytes, mediated by changes in the structure of the cell membrane. It is now clear that macromolecules from a variety of biologic sources can elicit proliferation in differ-

ent classes of lymphoid cells. These molecular stimulants, and their use in the elucidation of the mammalian immune response, have been extensively reviewed.[24,27] This discussion is focused on the cell kinetic aspects of lymphocyte proliferation *in vitro* in an attempt to show how such information can be used in the understanding of cell proliferation in lymphocytic lymphomas.

Pokeweed Mitogen–Stimulated B Cell Proliferation

The majority of human lymphocytic neoplasms originate in cells of the B lymphocyte lineage (i.e., cells producing and bearing on their surface immunoglobulin molecules). The proliferation and differentiation of T and B lymphocytes after exposure to the plant mitogen pokeweed mitogen (PWM) has been studied in great detail as a model of the humoral immune response. Some of the features of this model shed light on the appearances and behavior of B cell neoplasms.

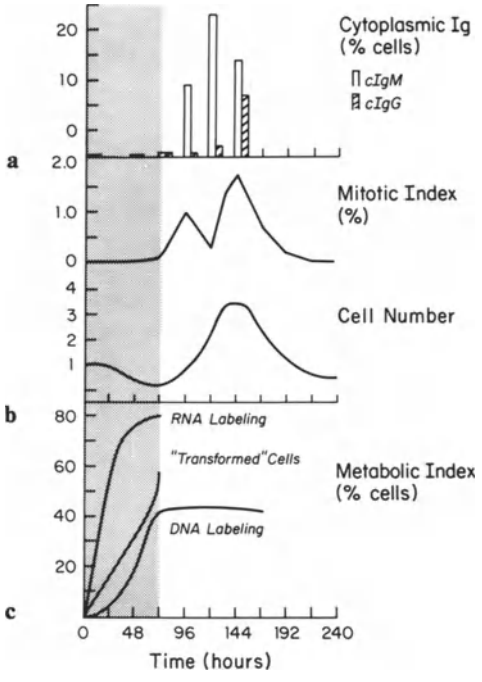

FIGURE 11–3 Lymphocyte transformation *in vitro:* events in PWM-stimulated cultures of human peripheral blood lymphocytes. 0 hours: time of addition of mitogen to the culture. **(a)** Development of intracytoplasmic immunoglobulin (cIg) in cultured cells. **(b)** Wave of cell proliferation after exposure to PWM, and bimodal increase in mitotic index, indicating two distinctive waves of cellular division. **(c)** Dramatic increase in RNA synthesis (^{3}H-uridine labeling), DNA synthesis (^{3}H-thymidine labeling), and appearance of the "transformed" morphologic phenotype in cultures upon the addition of PWM. Shaded area represents the pre-mitotic time interval in which "resting" cells are preparing for mitotic division by increase in cellular volume and new RNA and DNA synthesis.

Farnes et al., in 1964, reported that extracts of the plant *Phytolacca americana* (pokeweed) produced blastic transformation and mitosis of human mononuclear blood cells *in vitro.*[17] In a subsequent article, they reported large immature basophilic lymphocytes and plasmacytosis, as well as elevated serum IgM levels in two of their laboratory workers accidently exposed to the root extract.[2]

Blood lymphocytes cultured with PWM *in vitro* undergo a series of marked morphologic and metabolic changes resulting in proliferation of T and B lymphocytes, and in differentiation of the latter into immunoglobulin-synthesizing plasma cells and plasma blasts. B cell proliferation and differentiation in this system differ from that of an *in vitro* immune response in that they are not evoked by antigen and are polyclonal in nature (i.e., plasma cells of multiple immunoglobulin classes result). Despite these differences, the model has proven useful in explaining various immune phenomena and immunodeficiency states;[10,23,26] and if we accept a degree of cellular economy, it is reasonable to suppose that many of the mechanisms of subcellular activation, proliferation, growth control, and differentiation are similar in the *in vitro* model and the *in vivo* response of B cells to antigenic stimulus.

Metabolic "Activation" of Lymphocytes

The dramatic metabolic and morphologic events occuring in PWM-stimulated lymphocyte cultures were detailed by Chessin et al.,[7] whose findings are summarized in Figure 11–3c. Within 2 hours of exposure to PWM, new RNA synthesis is evident, increasing to a plateau at 48–72 hours. Morphologic "transformation," characterized by increased cellular diameter, decreased nuclear to cytoplasmic ratio, and cytoplasmic basophilia, is first detected at 16–18 hours of culture, reaching a peak by 66–78 hours, when 58% of cells display the "transformed" morphology. Beginning at 24 hours, and paralleling the increase in numbers of transformed cells, increased DNA synthesis is observed. By 72 hours, over 40% of cells in the cul-

ture have incorporated ^{3}H-thymidine. In summary, exposure to PWM triggers a profound series of metabolic events in susceptible cells, leading to accelerated RNA synthesis, increased cell size, and increases in DNA synthesis prior to cellular proliferation.

Proliferative Events

Yen and Stein studied the cell kinetics of PWM-stimulated proliferation in detail[65] and found that the stimulated wave of cellular proliferation was the sum of two distinct division cycles, the first incorporating a long lag prior to the entry of cells into mitosis, and the second shorter, with no lag period (Figure 11–3b). Upon completion of these division cycles, the cells could not be induced to further proliferation by the addition of fresh mitogen or culture medium. They concluded that PWM-stimulated cells execute two preprogramed division cycles ending in terminal quiescence in a G_0 (nonproliferative) state. This preprogramed limitation of cellular division might represent one mechanism by which cellular amplification in the immune response is regulated.

Terminal Differentiation of B Lymphocytes

Cell division is rare or absent in terminally differentiated plasma cells whose function is the production of specific antibody. Whereas B cells prior to immune stimulation bear antibody on the cell surface, such antibody is localized in the cytoplasm of mature plasma cells. In PWM-stimulated B cells, cytoplasmic immunoglobulin can be detected progressively only after the proliferative phase of the culture has begun. (Figure 11–3a).[60] During the quiescent terminal phase many of the cells have the appearance of plasma cells and plasma blasts.[35]

Coupling of Differentiation and Proliferation

As we have seen, cell proliferation and the appearance of differentiative markers (cytoplasmic immunoglobulin, plasmacytoid morphology) are closely linked in the *in vitro* model. Taken together, these findings support the hypothesis that a genetic program exists in human cells, tightly coupling limited cellular proliferation with differentiation of B cells to plasma cells. Such a program might represent one mechanism by which cellular amplification in the immune system is regulated. Errors in the reading of termination instructions in the program might result in the accumulation of lymphoid cells far in excess of the demands of the antigenic challenge, expressed as neoplasia.

Lymphocyte Transformation *In Vivo* and Lymphomas of Follicular Center Cell Origin

Proliferative processes similar to *in vitro* lymphocyte transformation occur in the germinal centers of normal lymph nodes in response to immune stimuli. A lymph node taken from a normal adult can be shown to contain a small fraction of cells in the mitotic cycle.[43] This fraction may increase considerably when tissue responding to immune stimulus is examined (see Figure 11–8), and proliferative activity is seen to be higher within lymph node germinal centers than in nearby interfollicular zones.[38,43] A large body of evidence indicates that germinal centers of lymphoid follicles are the site of B cell proliferation and differentiation. Lennart has summarized the evidence that germinal centers are the sites for transformation and multiplication of antigen-responsive clones.[7] He has termed the morphologic cell types appearing in germinal centers *centrocytes*—small or medium-size cells with notched, indented, or deformed nuclei, small nucleoli, and pale gray-blue cytoplasm —or *centroblasts,* with round nuclei, intensly basophilic cytoplasm, and medium-size nucleoli. Autoradiography shows proliferative activity and protein synthesis to be greater in centrocytes than in lymphocytes, with thymidine incorporation most frequent in centroblasts.[38]

Lukes and Collins also concluded that the transformation of B lymphocytes occurs within follicular centers and termed cells of these areas *follicular center cells.* They categorized small and large subtypes with variations in nuclear cleavage, and a larger cell type with basophilic cytoplasm, which they termed *immunoblast,* in recognition of its similarity to the blastoid cells of mitogen-stimulated cultures.[39] Lukes and his colleagues hypothesized that the various morphologic appearances of follicular center cells are expressions of different states in the process of lymphocyte transformation, and that malignant lymphomas of these cells may arise from a "block" or "switch on" of lymphocyte transformation, resulting in cytologic types of lymphomas resembling their normal counterparts both morphologically and functionally. Later, noting previous studies relating cell size to ^{3}H-thymidine uptake in lymphoid populations, and considering the relationship between the DNA content of folli-

cular center cells and their morphologic features, they proposed that cell cycle times become shorter as cells become larger. This observation extended the concept of "arrest" in lymphoid transformation to include *cell kinetic* states of the tumor cells as well as their morphologic features.[40] Further support for this concept was supplied by Shackney and Skramstad, who, in an elegant flow cytometric study, showed that neoplastic lymphoid cells become progressively larger as they traverse the cell cycle.[55]

In summary, cell size, thymidine uptake, and morphologic appearance are expressions of the cell kinetic states of both normal and neoplastic lymphoid cells. In addition, from information presently available, we can deduce the probable existence of a genetic program for coupled, self-limited proliferation and differentiation in B cell populations in which errors in "reading" at specific points could lead to arrest during the proliferative phases of the program. Uncontrolled amplification of cell populations at these points would lead to neoplastic expression. Furthermore, if the points in the program at which such errors occur are few in number, the discrete varieties of cellular appearances of lymphocytic lymphomas would be in part explained.

Cell Kinetics of Lymphocytic Lymphomas

Notation on the Present Classification Schemes for Lymphocytic Lymphomas

Much of the following discussion relates to the use of cell kinetic features of lymphoid neoplasms in the formulation of new classification schemes for these disorders, which are urgently needed by physicians caring for affected patients. Therefore, a short discussion of present histopathologic classification schemes, by way of introduction, is appropriate.

What characteristics are desirable in a taxonomic scheme for a group of neoplastic diseases? From a purely scientific viewpoint, such a classification should recognize the biologic similarities of the neoplastic cells and their normal counterparts in terms of their ontogeny, level of differentiation, and functional behavior. In keeping pace with advances in basic biologic knowledge, a progressive classification scheme should facilitate the application of new scientific findings to the understanding of the diseases and the formulation of improved therapies. From the viewpoint of the clinician, a classification scheme should be accurate, highly reproducible, and predictive of the natural history of the diseases and responses to specific therapies.

Since the late 1960s, the most widely used classification for non-Hodgkin's lymphomas, with slight modifications, has been that of Rappaport,[50] which is based on two morphologic criteria: overall pattern of neoplastic involvement of tissues on histopathologic sections, and cytomorphology of the neoplastic cells. The scheme does not address any biologic features of the neoplasms, except morphologic appearance, and its usefulness rests upon its ability to predict, on a statistical basis, the clinical outcomes in groups of affected patients.[1,29] However, when individual patients are considered, its predictive powers are considerably weaker. For instance, 20%–40% of patients with the advanced stages of "diffuse histiocytic lymphoma," a single category of the Rappaport scheme, fail to obtain a complete remission even with the best available therapy,[53,59] but another subset (20%–40%) attains prolonged disease-free survival and possibly cure.[42,52] These differences are not explained or predicted by the morphologic features of the tumors.

Consideration of other clinical features such as serum lactate dehydrogenase level at presentation,[34,54] sites and bulkiness of tumor involvement.[18,66] etc. have somewhat improved the precision with which we can predict clinical outcomes in each morphologic category; yet the usefulness of these refinements is diminished by an additional problem of purely morphologic schemes—that of reproducibility. Studies have demonstrated that general pathologists, using published criteria and reference fascicles, are unable to accurately classify these disorders by morphology in a substantial fraction of cases when their findings are reviewed by panels of reference pathologists.[16,31] Such panels have a higher rate of agreement among themselves, but this may in part be due to frequent cross-consultation and acceptance of certain assumptions by common agreement. To the extent that histopathologic interpretation remains a subjective skill, these weaknesses will persist despite frequent revisions of terminology. This creates a serious problem for clinical researchers who attempt to compare the efficacies of various treat-

ments in groups of patients treated at different institutions, since such comparisons require that each group be composed of patients with the same diseases.

More recently proposed histopathologic schemes have incorporated concepts derived from progress in the science of immunology after the Rappaport system was formulated. The Kiel classification,[37] proposed by Lennart, and the system of Lukes and Collins[39] take into account the phenomenon of lymphocyte transformation both *in vivo* and *in vitro*. They compare the morphologic and cell kinetic features of transformation with the appearances of both normal lymphoid cells and the neoplastic cells of certain lymphomas. In addition, they also correlate histopathologic findings with studies of the functional differentiation of the neoplastic cells along the known pathways of normal lymphoid cells. These classifications are a considerable advance toward a modern scientific

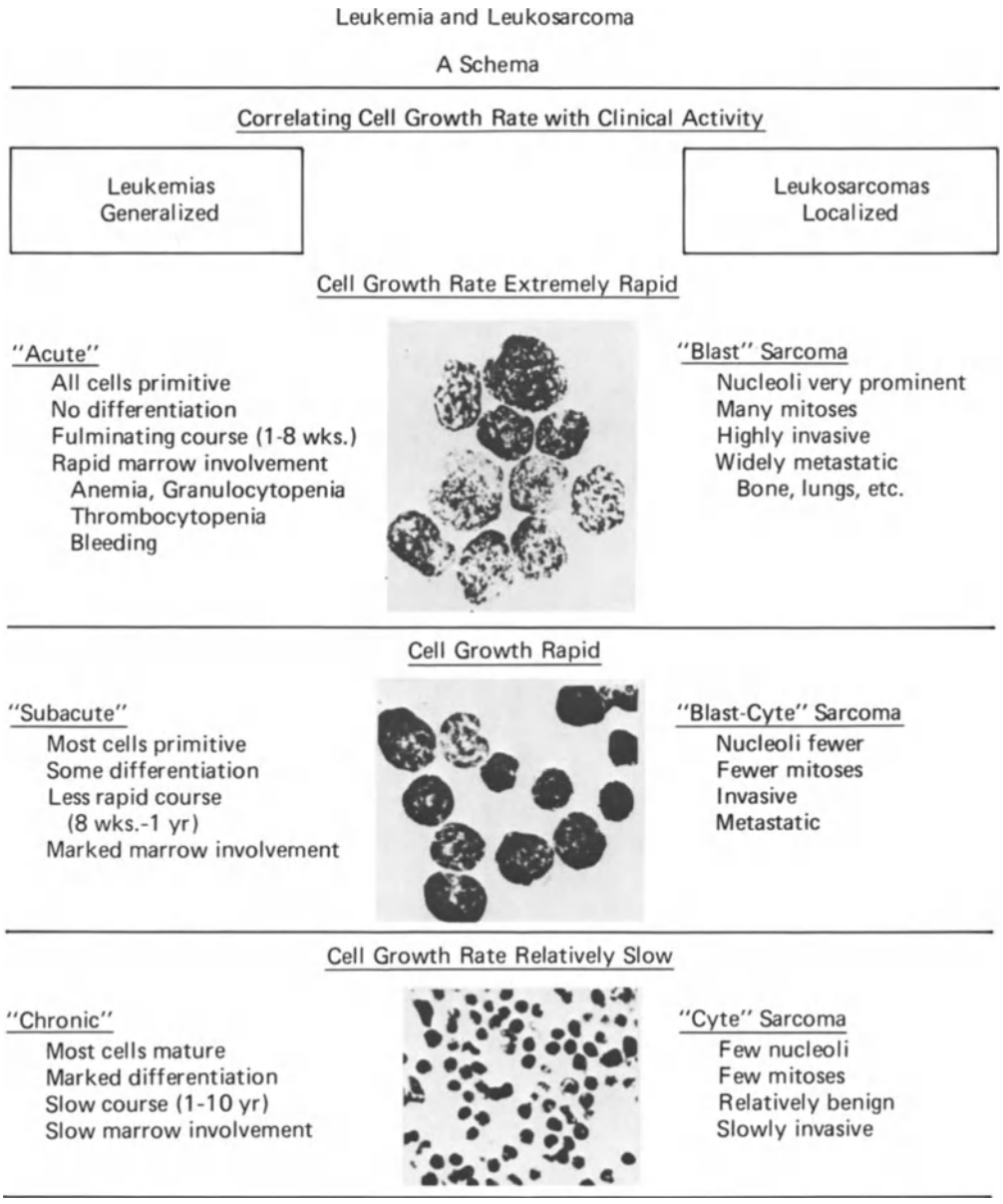

FIGURE 11–4 An early cytokinetic classification for hematopoietic tumors: poster by William Damashek (1949),[13] relating clinical disease manifestations with mitotic activity in leukemia and "leukosarcoma."

taxonomy for lymphomas, although their superiority to the Rappaport scheme, in terms of reproducibility and clinical application, remains unproven at this time. Without diminishing their contributions, it is fair to say that what is lacking in these schemes are quantitative diagnostic and prognostic criteria. Quantitative criteria, if reproducible and predictive of tumor behavior, would allow investigators in any institution or locale to determine precisely the type of disease under study, and to compare the results of parallel investigations or treatments with greater reliability. The practicing physician would gain confidence in his prognostication of the diseases and determination of treatment.

In the past two decades, a considerable amount of quantitative data regarding the *in vivo* growth characteristics of non-Hodgkin's lymphomas has been accumulated. For reasons that are unclear, these data have not been used by those attempting to formulate useful classifications for these disorders. In the subsequent discussion some of these data are summarized and it is shown how they may be useful in the design of improved classification schemes:

Cytokinetic Features of Lymphoid Tumors

It has long been recognized that tumors of the lymphoid system manifest a wide spectrum of clinical growth patterns and "aggressiveness." Dr. William Damashek, at a meeting of the American Medical Association in 1949, displayed the poster reproduced in Figure 11–4.[13] Referring to leukemias and "leukosarcomas," he attempted to clinically correlate cell growth rates with the cytologic appearances of the neoplastic cells and disease activity. His scheme defined three categories of leukosarcoma whose cellular appearances and associated diseases ran very different courses: The *blast* sarcoma form was characterized by relatively large cells, with prominent nucleoli and many mitoses. Its clinical behavior was described as "highly invasive" and "widely metastatic," killing the patient within 1–8 weeks. A second type, the *blast-cyte* sarcoma, had cells with fewer nucleoli and mitoses, an invasive and metastatic behavior, and a less rapid clinical course of 8 weeks to 1 year. The third category, *cyte* sarcoma, had rather small cells, few nucleoli or mitoses, a slowly invasive behavior, and a clinical course that could run for years before death. Damashek's keen observations were clinical and morphologic; and in the ensuing years, a body of quantitative knowledge regarding the *in vivo* growth of lymphocytic lymphomas was generated which confirmed his impressions of the varying patterns of cellular proliferation in these diseases.

Table 11–1 summarizes some important proliferative parameters for lymphocytic lymphomas

TABLE 11–1 *In Vivo* Growth Characteristics of Lymphocytic Lymphomas in Man

Observed Volume Doubling Time (days)	Mean Cell Cycle Time (hours)	Growth Fraction (%)	Tumor Type	Reference
2.8	26	100	Burkitt's lymphoma	28
30	66	32	Lymphosarcoma	45
35	80	31	Reticulum cell sarcoma	45
70	116	15	Reticulum cell sarcoma	45
105	159	11	Reticulum cell sarcoma	45
115	194	14	Reticulum cell sarcoma	45
400	264	5	Lymphosarcoma	45
	60	60	Lymphosarcoma	56
	100	100	Lymphosarcoma	56
	59		Reticulum cell sarcoma	49
	91		Lymphosarcoma	8

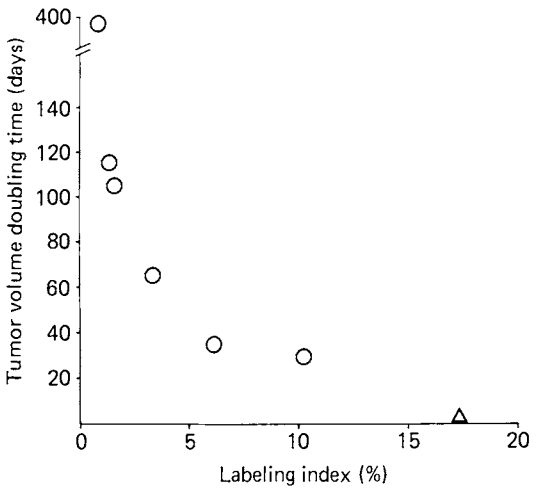

FIGURE 11–5 *In vivo* cell kinetics of lymphocytic lymphomas: relationship between ^{3}H-thymidine labeling index and measured tumor volume doubling time. ○ Nishikori and Shirakawa[45]; △ Iverson et al.[28] (From Hansen et al.,[24a], with permission.)

derived from *in vivo* studies in man using parenteral injections or infusions of ^{3}H-thymidine. The wide range of proliferative behavior in these tumors is evident, with macroscopic tumor volume doubling times as short as 2.8 days, to as long as 400 days, mean cell cycle times from 26 to 264 hours, and growth fractions from 5% to 100%.[8,28,45,49,56]

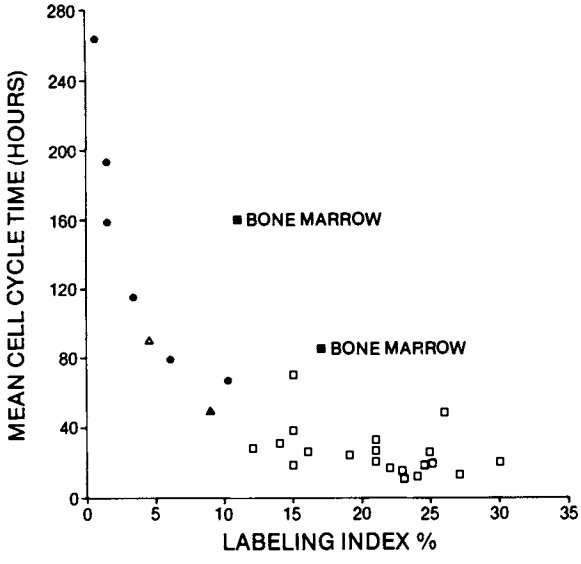

FIGURE 11–6 *In vivo* cell kinetics of lymphocytic lymphomas: relationship between ^{3}H-thymidine labeling index and mean cell cycle times of tumor cell populations. △ Clarkson et al.[8]; □ Iverson et al.[28]; ● Nishikori and Shirakawa[45]; ▲ Peckham and Steel[49]; ■ Sheehy et al.[56] (From Hansen et al.,[24a] with permission B: Marker and kinetic studies in non-Hodgkin's lymphoma. Am J Med 71:107–123, 1981, with permission.)

S-Phase Fraction as a Measure of Tumor Growth

Although very useful, the time, technology, and expense of *in vivo* studies precludes their application to large numbers of patients, and therefore a simpler, more widely applicable method of estimating tumor growth patterns in individuals would be very useful. Such a method appears to be the measurement of the S-phase fraction by simple *in vitro* techniques such as autoradiographic LI or flow cytometry. Figures 11–5 and 11–6 show the relationship of short-duration ^{3}H-thymidine labeling to tumor volume doubling times and mean cell cycle times measured *in vivo* in lymphocytic lymphomas. There is an approximately hyperbolic relationship between volume doubling time or mean cell cycle times and LI; higher LI's are found in rapidly growing tumors with short mean cell cycle times, and lower LIs in slowly growing tumors with longer mean cell cycle times. The growth fraction, shown in Figure 11–7, appears to increase linearly with the LI. In some cases the LI has been used to calculate growth fraction (GF) using the equation

$$GF = \frac{LI}{LI_{(P)}}$$

where LI_P is the labeling index of proliferating cells. The apparent linearity of this relationship when the measured values are plotted suggests that the LI of the *proliferative* fraction is constant within all the lymphoid tumors measured, the difference in growth fraction being accounted for by varying proportions of nonproliferating cells. Sasaki and Sakka have also found correlations of LI mean population cell cycle time, growth fraction, and tumor doubling time in solid tumors.[51] Hence the LI appears to satisfy the requirement for a simple *in vitro* test that can be used to estimate other tumor growth parameters in large numbers of human lymphocytic lymphomas.

Labeling Index Distribution in Lymphocytic Lymphomas

Many investigators have studied the LI in lymphoid neoplasms and found a very broad distribution for this parameter, in keeping with the spectrum of clinical tumor growth seen in these

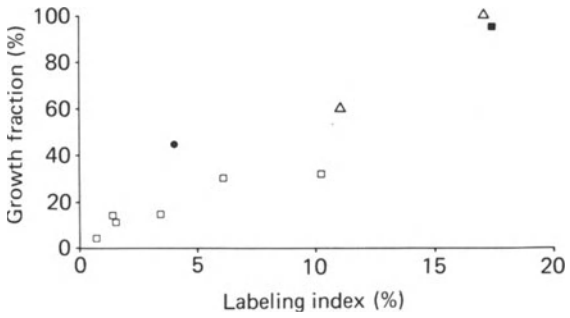

FIGURE 11–7 *In vivo* cell kinetics of lymphocytic lymphomas: relationship between ^{3}H-thymidine labeling index and the *in vivo* growth fraction of tumor cells. □ Nishikori and Shirakawa[45]; ■ Iverson et al.[28]; △ Sheehy et al.[56] (From Hansen et al.,[24a] with permission.)

diseases.[9,36,37,48,58] Figure 11–8 shows the distribution of LI values seen in 325 cases of involved tissues from patients with lymphocytic lymphomas studied at the Memorial Sloan-Kettering Cancer Center, and a similar distribution for 35 benign, but hyperplastic lymph nodes. The range for tissues involved by lymphoma was 0 to 58%, with a median value of 4%. The median value for nonneoplastic cases was similar (5%), with a range of 0.1%–27%. Over 90% of values for neoplastic tissues fell within the range seen in benign tissues, and therefore the LI alone could not in general be used to determine the neoplastic nature of the tissue. There is some relationship between the LI and the histopathologic appearance of the involved tissues in the Rappaport classification. This is shown in Figure 11–9, in which the LI measurements are shown for each Rappaport subtype. Of note is the wide range of values that can be observed in each of these histopathologic categories except nodular poorly differentiated lymphocytic and diffuse well-differentiated lymphocytic, emphasizing the weak correlation of tumor growth rates and histopathologic appearance in this scheme.

Cell Volume as a Kinetic Parameter in the Lymphocytic Lymphomas

Histopathologic classifications of lymphocytic lymphomas recognize cell size, as measured or estimated on stained tissue sections, as an important prognostic feature.[14] In the section, the relationship of cell size to kinetic behavior in lymphoid cells was outlined. Braylan and his colleagues quantified cell size in neoplastic and nonneoplastic lymphoid populations using a method that measures the individual volumes of large numbers of individual cells in tissue suspensions.[6] In this technique, electronic particle sizing (Coulter sizing),[33] the cells are suspended in an electrically conductive medium and are caused to flow through a tiny aperture separating conducting electrodes. The current flow between the electrodes is momentarily decreased as a relatively nonconductive cell passes through the aperture, partially obstructing it. The magnitude of this decrease in current is a function of the volume of conductive fluid displaced by the cell. Hence a signal is generated reflecting the volume of each cell as it passes the

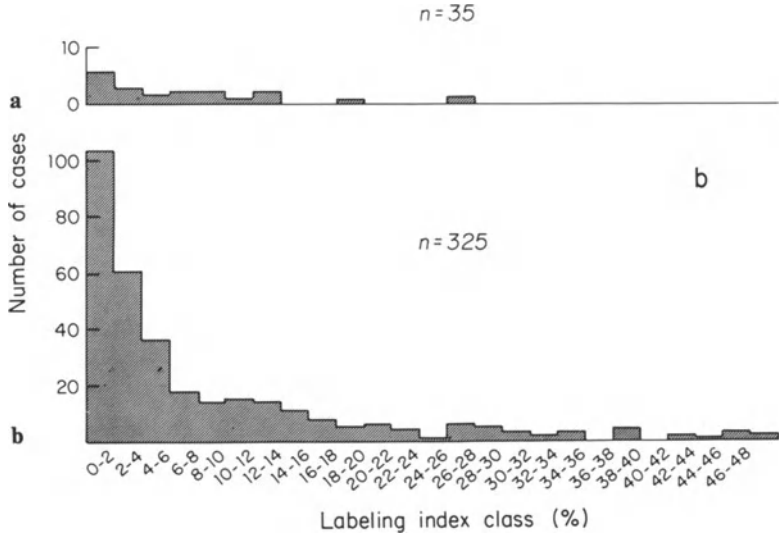

FIGURE 11–8 Frequency distribution of labeling index in lymphocytic lymphomas: measurements of ^{3}H-thymidine labeling index. **(a)** 35 cases of hyperplastic lymphadenopathy. **(b)** 325 cases of lymphocytic lymphomas.

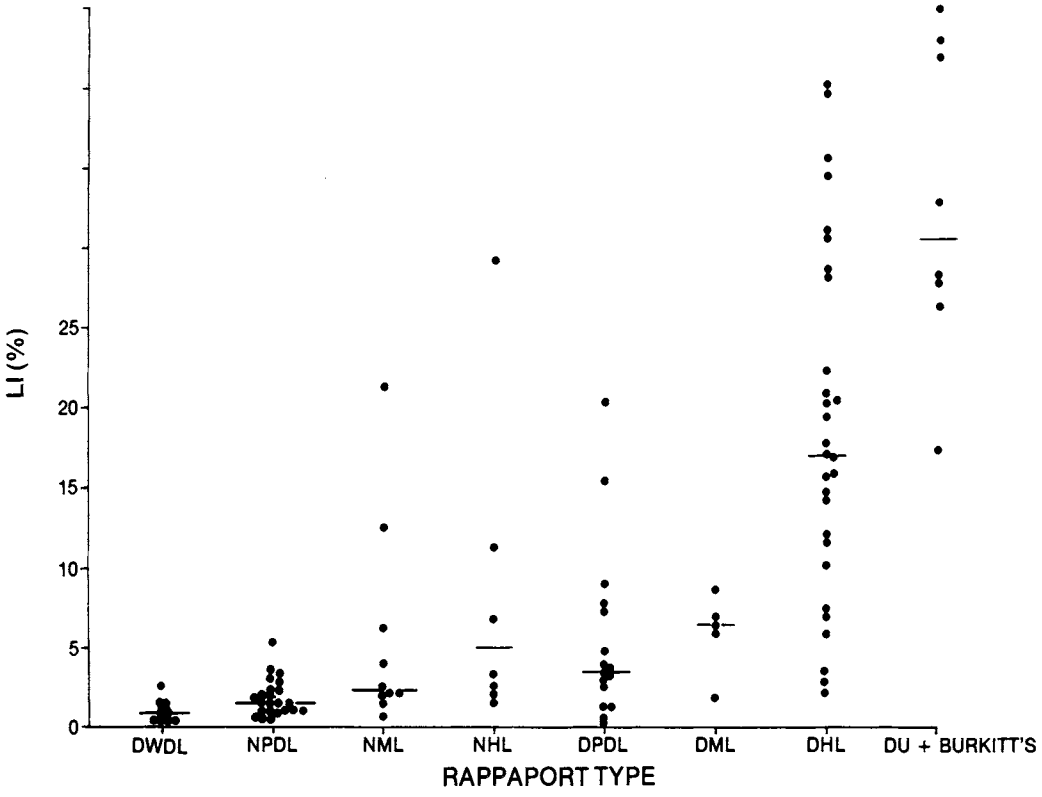

FIGURE 11–9 Labeling index and histopathology: distribution of ^{3}H-thymidine labeling index values for various histopathologic lymphoma subtypes according to the classification of Rappaport. DWDL, diffuse well-differentiated lymphocytic; NPDL, nodular poorly differentiated lymphocytic; NML, nodular mixed histiocytic–lymphocytic; NHL, nodular histiocytic; DPDL, diffuse poorly differentiated lymphocytic; DML, diffuse mixed histiocytic–lymphocytic; DHL, diffuse histiocytic; DU, diffuse undifferentiated. (From Hansen et al.,[24a] with permission.)

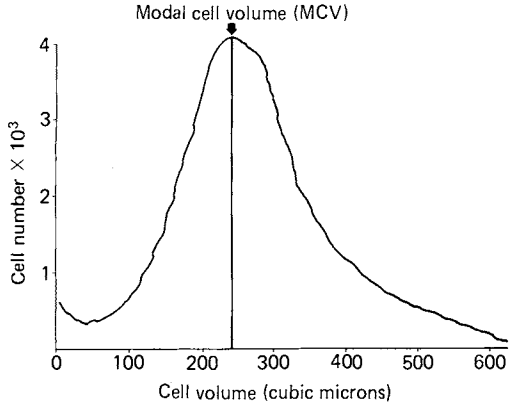

FIGURE 11–10 Coulter cell sizing method: cell volume histogram generated from a cell suspension derived from a lymph node involved by diffuse poorly differentiated lymphocytic lymphoma. Most frequently found cell volume (histogram mode) is termed the "modal cell volume" for the population.

aperture. Such measurements can be performed rapidly on large numbers of cells and a histogram of cell volume distribution in the population is generated. The mode of this histogram is determined and termed the "modal cell volume" (MCV) for the tissue analyzed. An example of such a histogram is shown in Figure 11–10.

As in the case of LI there is a wide variation in the range of MCVs seen in lymphocytic lymphomas, with values from 100 to 718 μ^3 reported by Braylan et al. In Figure 11–11 the frequency distribution of MCV values is shown for 326 tissue suspensions from involved tissues of patients with lymphocytic lymphomas, and 34 cases of nonneoplastic lymphadenopathies at Memorial Sloan-Kettering Cancer Center. The maximum value seen in the latter group was 260 μ^3. MCVs greater than this value were found only in neoplastic tissues, although some very large cells were present in each tissue examined. Thus the presence of large cells is not a criterion for malignancy, although

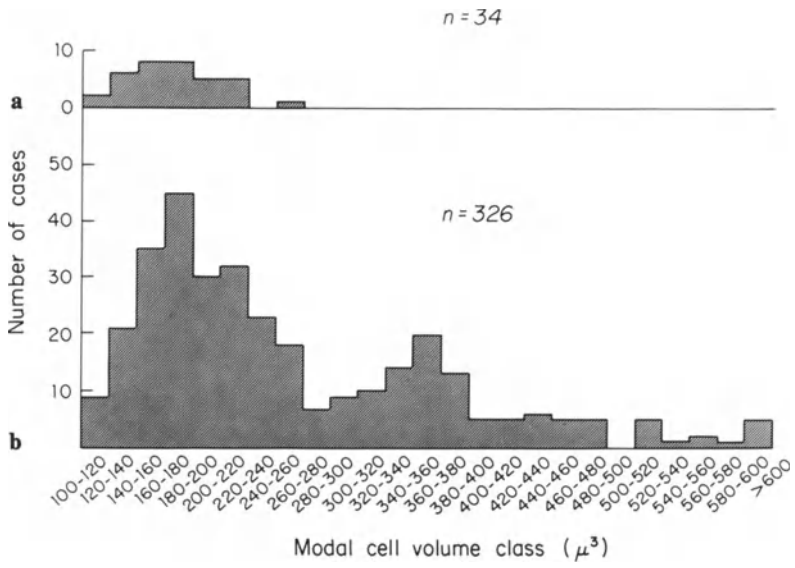

FIGURE 11–11 Frequency distribution of modal cell volume in lymphocytic lymphomas. **(a)** 34 cases of hyperplastic lymphadenopathy. **(b)** 326 cases of lymphocytic lymphomas.

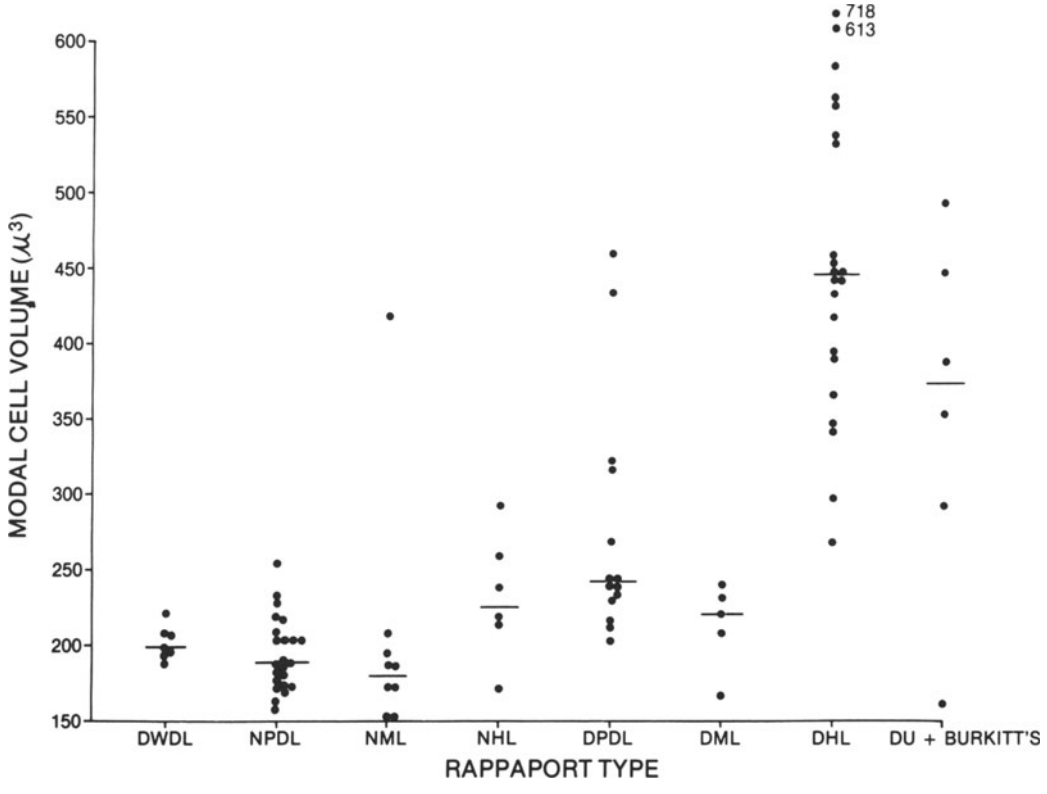

FIGURE 11–12 Modal cell volume and histopathology: values for various histopathologic lymphoma subtypes of the Rappaport classification. See Figure 11–9 legend for definition of abbreviations. (From Hansen et al.,[24a] with permission.)

certain size distributions are found only in neoplastic tissues.

In Figure 11–12, the MCVs for various types of lymphocytic lymphomas are shown according to the Rappaport type of the tissue studied. Again, there is a loose relationship of MCV with histopathologic appearance, and a large range of variability within the diffuse categories (with the exception of diffusive well-differentiated lymphocytic).

Cell Kinetics and the Outcome of Therapy in Lymphocytic Lymphomas

Neoplastic Cell Growth Rate

With the exception of documented localized disease, the majority of patients with these diseases are not cured by any currently available treatment modality. The typical course of events is one of induction of remission (partial or complete) with radiotherapy or chemotherapy, followed by relapse and progressively shorter periods of disease control, until the toxicities of therapy induce morbidity of the same or greater magnitude as the disease itself. At this juncture the patient succumbs to either or both of these morbidities.

Clinical oncologists have long recognized that the increment of reduction in relapsing tumors becomes progressively smaller with repeated applications of the same chemotherapeutic agents. This has been ascribed to the emergence of "drug-resistant" populations analogous to those seen in microbial populations during antibiotic therapy. Drug resistance appears to be a major factor in our inability to control neoplastic cell growth for periods similar to the normal life span of affected individuals.

The number of drug-resistant cells in a tumor cell population may be independent of cellular proliferation rate prior to treatment. However, there is evidence, based on mathematical modeling, to suggest that the emergence of resistance during attempts to control tumors with chemotherapy may be highly dependent upon this factor. Goldie and Coldman have proposed a model relating the drug sensitivity of tumors of various sizes to their rate of spontaneous mutation to resistance.[22] Their model assumes that the probability that a resistant clone of tumor cells will arise is a function of tumor size and the rate of spontaneous mutatation to resistance. In Figure 11–13 the calculated probability of the existence of totally resistant cells is shown for various overall tumor sizes and rates of spontaneous mutation to resistance. The latter parameter (α) is expressed as mutation to resistance *per cell generation.*

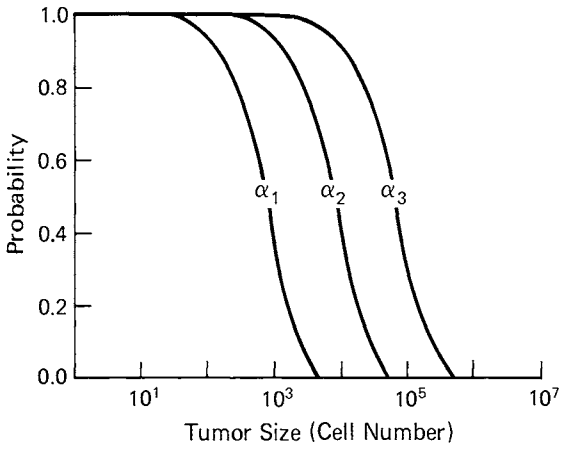

FIGURE 11–13 Goldie-Coldman resistance plot: plot of the probability of finding no totally drug-resistant cells in tumors of varying size (cell number) for varying rates (α) of spontaneous mutation to resistance per cell generation ($\alpha_1 > \alpha_2 > \alpha_3$). Tumors with cell numbers above the break points in the curves would not be curable by the agents to which resistance has developed. (From Goldie and Coldman,[22] with permission.)

For any spontaneous mutation rate to resistance, there is a sharp increase in the probability of a completely resistant population as tumor size increases above a threshold cell number. Since, as stated, our current treatment modalities do not eradicate tumor in most cases of disseminated lymphocytic lymphoma, we may conclude that these diseases frequently present with tumor burdens that are beyond the break point in the curves (i.e., contain "totally resistant" phenotypes). The survival of the host in most cases will then depend on the rate at which the *resistant* clones emerge to produce clinically evident tumors during antineoplastic therapy.

Let us consider how cell kinetic parameters of lymphoid tumors affect these factors: The kinetic analysis of neoplastic growth *in vivo* is complex, being the product of a large number of variables. For example, cellular growth rates and growth fractions vary within tumor masses due to variations in oxygen and nutrient supply, and large numbers of cells may be lost from the tumor population due to the production of defective daughter

cells in many mitotic divisions. Despite these difficulties, mathematical models of tumor growth have provided useful approximations of normal and neoplastic cell proliferation *in vivo.*[61] For the purposes of the present analysis, a series of simplifying assumptions are made in modeling the proliferation of resistant tumor cells within a lymphoma population *in vivo;* the approximate nature of the model is acknowledged attempt to show how the cell kinetic parameters of the neoplastic populations may affect the success or failure of treatment with antineoplastic drugs.

Net Gain of Resistant Cells per Unit Time

Let us assume that the cell kinetic parameters of the resistant population are similar to those of the tumor as a whole and are unaffected by therapeutic interventions, and that the proliferative fraction follows simple exponential growth kinetics. In this situation the number of resistant tumor cells (R) is given by the relation

$$R = R_0 \exp(kt)$$

where R_0 is the number of resistant cells at time $_0$ (onset of treatment); k is the growth constant ($\ln 2/T_c$); and T_c is the mean cell cycle time of the population. Then, after any subsequent interval Δt between cytotoxic treatments, the number of resistant cells in the tumor will be

$$R = R_0 + \Delta R = R_0 \exp\left(\ln 2 \frac{\Delta t}{T_c}\right)$$

and the net gain in resistant cells is

$$\Delta R = R_0 \left\{\exp\left(\ln 2 \frac{\Delta t}{T_c}\right) - 1\right\}.$$

The relationship between the mean cell cycle time and the number of resistant cells under these circumstances is plotted in Figure 11–14, showing that for any constant time interval Δt, the number of resistant cells will increase exponentially as mean cell cycle time becomes shorter. If Δt is the interval between treatments, the emergence of clinically important resistant tumor will occur more rapidly in tumors with short mean cell cycle times in the resistant population. This is especially true if $\Delta t/T_c$ is large, i.e., when treatment intervals are much larger than the mean cell cycle time of the resistant population. This is the situation in the chemotherapy of most human lymphocytic lymphomas, where Δt is usually on the order of 3–4 weeks. This interval is usually fixed by the requirement for recovery of normal hematopoietic cells between treatments (Table 11–1).

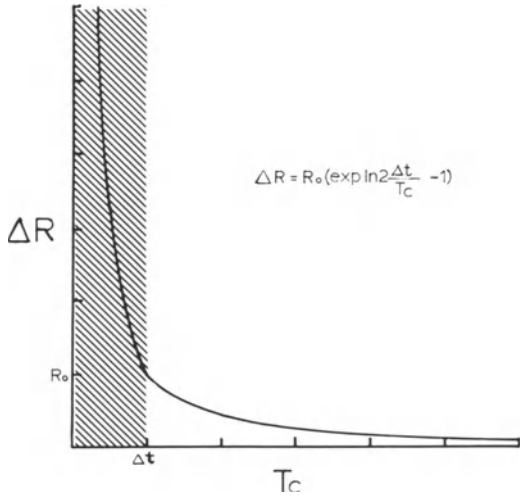

FIGURE 11–14 Mean cell cycle time as a determinant of drug resistance: increase in totally resistant cell phenotypes (ΔR) in any constant treatment interval Δt, in a tumor of initial size R_0, for varying mean cell cycle times of the neoplastic population. In the shaded area $\Delta t > T_c$, where ΔR increases rapidly with decreasing cell cycle times.

Rate of Spontaneous Mutation to Resistance After Treatment

The theory of Goldie and Coldman[22] is a static one, in that it assesses the development of drug resistance independently of cellular proliferation rates. However, in their model, which is based on previous studies of antibiotic resistance in bacteria, the rate of spontaneous mutation to resistance (α) is given by

$$\alpha = \frac{n}{g} \qquad (11\text{–}1)$$

where n is the number of spontaneous mutations to resistance, and g is the number of generations. If this assumption is true, then we can adapt the model to simple exponential cell growth during the treatment intervals of chemotherapy. Since in simple exponential growth,

$$g = \frac{t}{T_c} \qquad (11\text{–}2)$$

we can substitute the right-hand term in equation

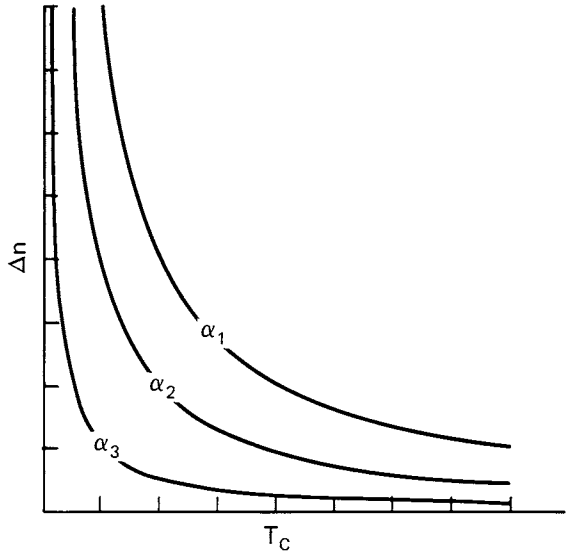

FIGURE 11–15 Mean cell cycle time and the accumulation of mutations to resistance: plot of the number of new spontaneous mutations to resistance in a tumor population during any treatment interval Δt for varying spontaneous mutation rates (α) and mean cell cycle times (T_c) of the neoplastic population (n).

11–2 for g in equation 11–2, and rearranging terms we obtain

$$n = \frac{\alpha t}{T_c}$$

and in any time interval Δt,

$$\Delta n = \frac{\alpha}{T_c}$$

i.e., the number of spontaneous mutations to resistance in any time interval is an inverse function of cell cycle time, and the *rate of accumulation of new mutations to resistance* is greater in rapidly proliferating cell populations. In Figure 11–15 this relationship is shown graphically for different spontaneous mutation rates to resistance.

If the assumptions of this simple model represent approximations of *in vivo* events in human lymphocytic lymphomas, we may conclude that during the chemotherapy of a tumor that contains resistant cells at the onset of treatment, both the number of resistant cells with time, and the rate

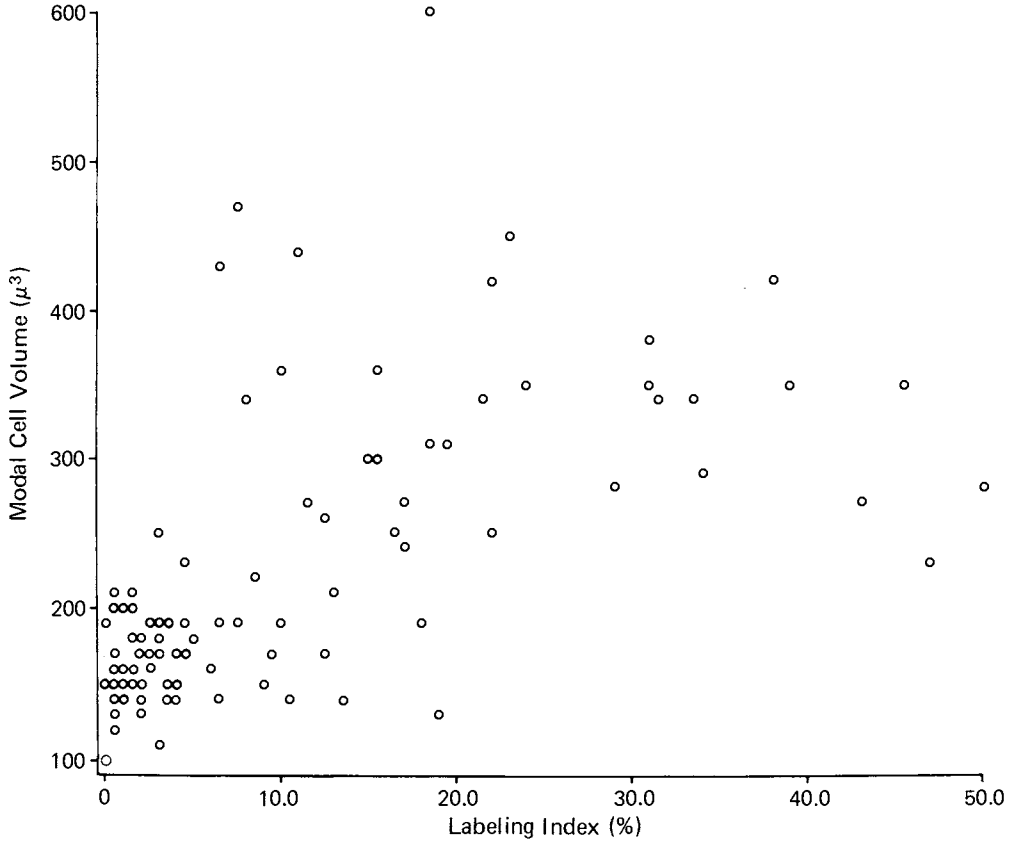

FIGURE 11–16 Simultaneous cell kinetic and cell volumetric measurements: two-dimensional plot of labeling index and modal cell volume measurements determined on 90 specimens of cells derived from the involved tissues of patients with lymphocytic lymphomas.

of appearance of new resistant clones will be increased in populations with shorter mean cell cycle times. In these tumors, treatment failure and death of the host will be evident at an earlier time during therapy.

Labeling Index and Prediction of Therapeutic Outcome

We have seen that higher LIs are associated with shorter mean cell cycle times in lymphocytic lymphomas, and how the latter parameter may predict early treatment failure. There is ample evidence to suggest that the LI of tumor cells is an important prognostic parameter in human neoplastic diseases. Because of its direct relationship to various parameters of tumor growth, the prognostic significance of the LI has been investigated in several types of human neoplasms. Straus and Moran, using *in vivo* cell kinetic methods, studied the significance of the LI in breast cancer tumors, and suggested that higher tumor cell LIs are associated with a poorer prognosis.[63] Gentili et al. recently reported that in premenopausal breast cancer patients with negative axillary lymph nodes, postsurgical relapse rates were significantly higher for those patients whose tumors had *in vitro* LIs higher than the median value of 4.6% in their series.[20] Others have reported an adverse impact of high initial tumor cell LI on remission duration in acute nonlymphocytic leukemia[12,25,64] and on survival in multiple myeloma.[15]

Costa et al. measured the labeling index in 88 cases of untreated lymphocytic lymphomas and examined the remission rates and survivals of patients whose tumors had labeling indices above or below the median value for all cases measured.[11] They found that both remission rates and overall survivals were significantly higher in patients whose tumor LIs were below the median, offering

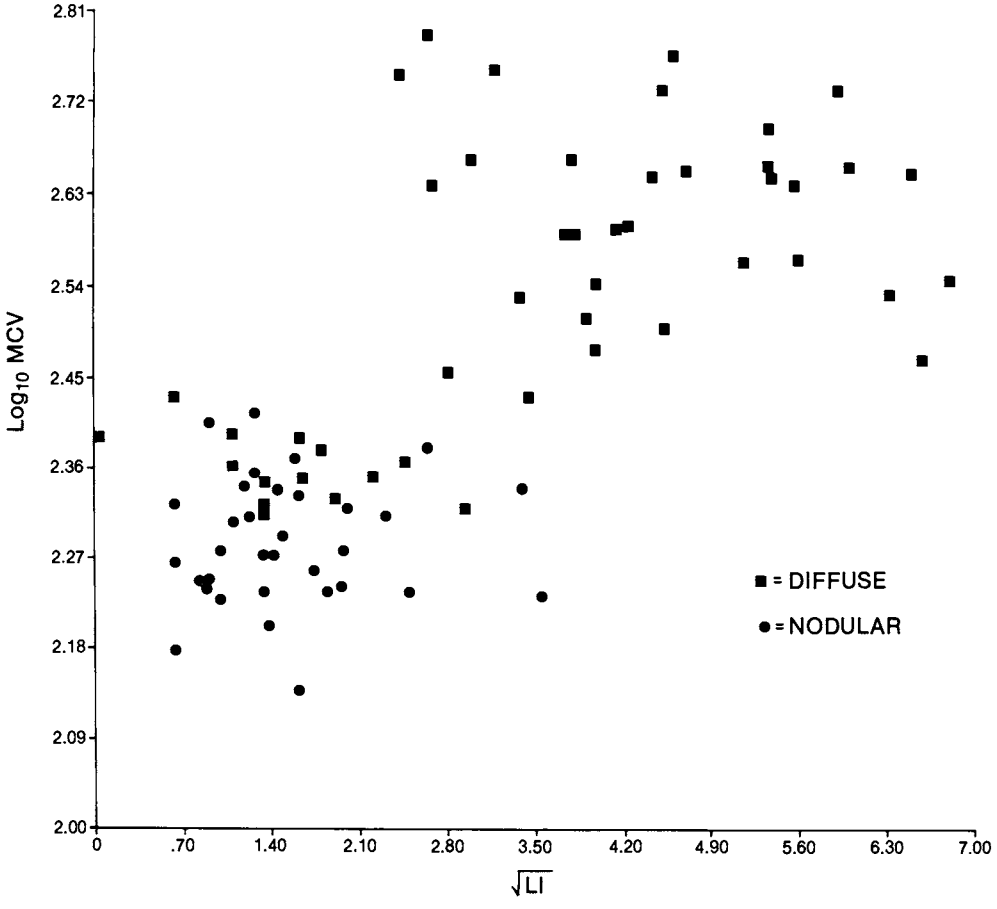

FIGURE 11–17 Kinetic–volumetric map: after kinetic and volumetric data have been normalized by means of simple transformations. Two-dimensional plot of 80 cases of lymphocytic lymphomas using transformations (square root for labeling index, logarithim for modal cell volume). (From Hansen et al.,[24a] with permission.)

direct evidence of the prognostic value of this measurement in the lymphocytic lymphomas.

Cell Kinetic Parameters and the Classification of Lymphocytic Lymphomas

We have shown that cell size and LI relate to the cell kinetic states of lymphoid cells, and how such states reflect the growth rates of lymphoid tumors. How can such information be used to help formulate better classification schemes?

In Figure 11–16 a two-dimensional plot of the LI and MCV measured simultaneously in 90 cases of lymphocytic lymphomas is shown, with each case represented by (LI, MCV) as an (x,y) pair. A similar data set is shown in Figure 11–17 after simple mathematical transformations are used to normalize the data on each axis. We have termed such a plot a "kinetic–volumetric map." When the data are thus transformed, two large clusters of points are apparent, one cluster composed of small cells with low LIs and the other of larger cells and higher LIs. If an area is determined on the map for each set of points belonging to a Rappaport classification subtype the map in Figure 11–18 results. Nodular lymphomas, a "good-prog-

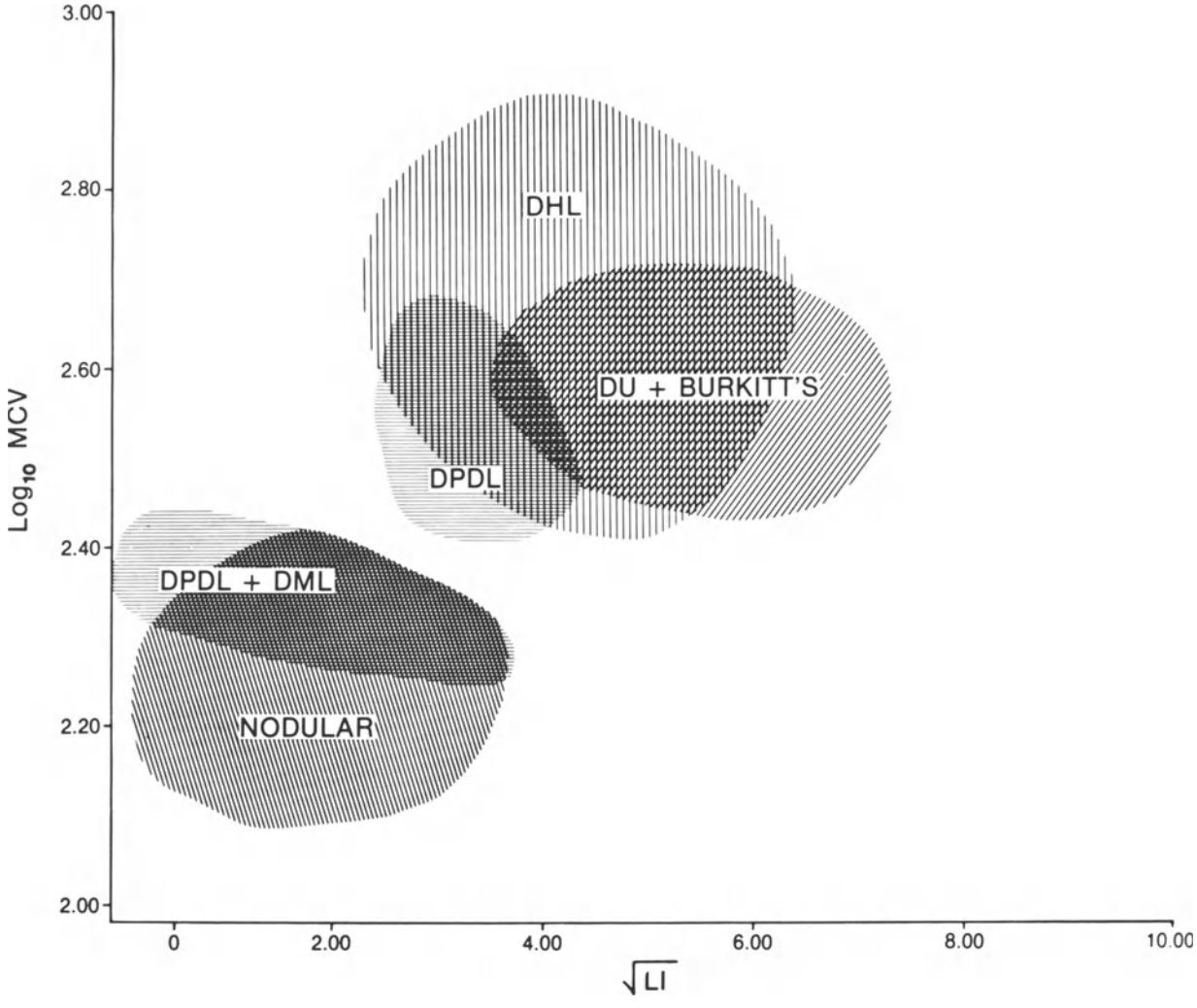

FIGURE 11–18 Kinetic–volumetric map of lymphocytic lymphomas: areas are localized for histopathologic types of lymphocytic lymphoma tissues whose labeling index and modal cell volume data have been plotted. Various shadings define areas in which the points for tissues of each Rappaport histopathologic type are located. See Figure 11–9 legend for definition of abbreviations. (From Hansen et al.,[24a] with permission.)

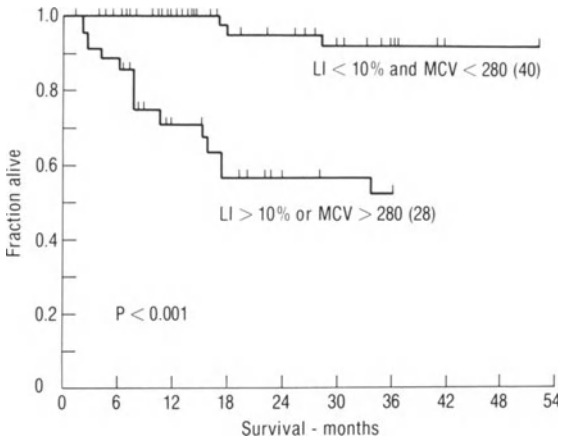

FIGURE 11–19 Kinetic–volumetric map area and patient prognosis: actuarial survival curves for patients whose lymphoma tumor cells have labeling indices less than 10% and modal cell volumes less than 280 μ^3 (kinetic–volumetric type I) and those whose tumors have higher values for either parameter (kinetic–volumetric type II). The difference in survival is highly significant by the log rank statistical test.

nosis" group in the Rappaport system, are found only in the lower cluster. The upper cluster is composed exclusively of diffuse categories, whose point loci partially overlap one another. Some diffuse lymphomas, however, may be found in either category. We have termed lymphomas whose (LI, MCV) loci fall within the lower and upper clusters "kinetic volumetric" types I and II, respectively.

Preliminary analysis of survival data has shown this method of characterizing lymphomatous tissues to be clinically predictive of some aspects of disease behavior in affected individuals. The survival curves of patients with kinetic–volumetric type I and II lymphomas of B cell origin are compared in Figure 11–19. The difference in overall survival at 36 months is highly significant. Analysis of more cases and longer follow-up will be necessary to determine whether subdividing clusters into discrete areas will identify further prognostic categories; however, the advantages of such a classification scheme are already apparent: it utilizes precise, highly reproducible, and easily measured parameters of the neoplastic cells; the parameters reflect important biologic features of the tumors; and quantitative measurements can be subjected to a wide variety of statistical analyses. It is hoped that further studies in this area will lead to more precise and useful classification schemes for these disorders.

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CHAPTER 12

Infections in Lymphoma Patients

Merril Gersten and Donald Armstrong

Infectious complications remain a significant cause of mortality in patients with lymphatic neoplasms.[6,40] The high incidence of infection reflects both immune alterations inherent in the diseases and those engendered by antineoplastic therapy. These patients are at risk for infection caused not only by organisms normally considered to be virulent pathogens, but also by less virulent organisms that often are a part of the normal flora. Infections of the latter type are often more troublesome to treat because the organisms involved are frequently more antibiotic resistant than the usual pathogens.

It is possible to some extent to predict the organisms most likely to be responsible for infection based on the patient's most prominent immune defect. The four main categories of immune defects are

1. Impaired neutrophil defense, due most often to low circulating numbers of polymorphonuclear leukocytes following chemotherapy, or due to bone marrow invasion by lymphoma
2. Defective T lymphocyte–mononuclear phagocyte function, usually associated with prolonged therapy, occasionally a consequence of the lymphoma itself
3. Abnormal gamma globulin function, most commonly due to hypogammaglobulinemia associated with chronic lymphocytic leukemia (CLL) and multiple myeloma, but also seen with prolonged chemotherapy
4. Splenic dysfunction following splenectomy for massive spleen enlargement, or due to invasion by neoplastic lymphoma cells.

Most of the non-Hodgkin's lymphomas are, like CLL, of B cell origin and tend to be associated with abnormal humoral immunity. A notable exception is lymphoblastic lymphoma (formerly called lymphosarcoma of childhood), which appears to be of T cell origin.[10]

Immune defects are not always so isolated as the preceding description suggests; frequently the picture is complicated by multiple factors such as iatrogenic immunosuppression due to antineoplastic chemotherapy, radiotherapy, and (staging) splenectomy. Adrenocorticosteroids produce both a lymphocytopenia and a monocytopenia, but significantly impair only the efferent (monocyte–macrophage) arm of cell-mediated immunity.[19,65] The neutrophil leukocytosis seen with steroid treatment is associated with a decreased egress of cells from the blood into areas of inflammation, accounting, in part, for the reduction in physical signs of inflammation in infected patients receiving steroids. The effect of cytotoxic drugs on immune function is influenced by factors such as dose and scheduling of drug or drug combinations;[8] however, the most profound and common effect is a drastic reduction in the number of circulating neutrophils. Radiotherapy initially reduces numbers of both circulating B and T lymphocytes. Recovery of B cells begins shortly after completion of radiotherapy and is usually complete within 2 years; but T cell numbers and certain in vitro parameters of T cell function may remain suppressed for as long as 8–10 years after irradiation.[24]

The spleen is important in the production of early (IgM) opsonins, and in the sequestration of particulate antigen early in infection; and its macrophage population significantly contributes to

the body's handling of intracellular pathogens. For these reasons, splenectomy compromises the host's immune system in more than one sphere, and in particular, puts the patient at risk for overwhelming postsplenectomy infection, a fulminant bacteremia caused by encapsulated organisms (*Streptococcus pneumoniae, Hemophilis influenzae* and *Neisseria meningiditis*) which may lead to death within 10–18 hours after onset of symptoms.[17,18,37,42]

Finally, the patient with a lymphatic neoplasm may be further predisposed to infection by general and local factors that interrupt the integument (chemotherapy-induced mucosal or gastrointestinal ulcers, bladder or intravenous catheters) or introduce microorganisms through intravenous infusions (blood product, fluids). The organisms that most commonly take advantage of the various types of immune defects are listed in Table 12–1.

Signs of Infection and Clinical Sepsis

Fever is generally the hallmark of an infection, however, in dealing with patients who have a lymphatic neoplasm, it is important to remember that fever may occur in the absence of infection and be due to the underlying disease, drug reaction, or transfusion reaction, and infection may occur in the absence of fever. The latter circumstance is most often seen in patients who are severely ill and debilitated, in the elderly, in patients receiving adrenocorticosteroids, or in patients infected with certain organisms, such as *Cryptococcus neoformans,* which may not evoke a febrile response. Early occult sepsis in these patients may be heralded by hyperventilation alone, or may be suggested by an unexplained leukocytosis, leukopenia, thrombocytopenia, tachycardia, hypotension, or subtle mental changes.

When fever does occur in these patients, it should first be regarded as infectious and should prompt intensive investigation for the source. If a source can be detected, the responsible organism can frequently be identified and appropriate therapy administered. Such therapy may include a drainage procedure when a closed-space infection is found (e.g., subcutaneous abscess, pulmonary abscess behind an obstruction), in addition to the antimicrobial selected on the basis of known or presumed sensitivities. If the patient is neutropenic (leukocyte count below 2000/mm^3) or clinically convincingly septic, then empiric therapy should be started. In some cases in which the leukocyte count is normal, in the absence of a proven source clinically or by culture, antimicrobial treatment may be withheld. Rapid investigation with appropriate cultures and diagnostic procedures such as x-rays and scans should be done. Experience has shown that "blind" therapy diminishes the normal flora, permitting overgrowth of resistant organisms, and also hampers further efforts to find the source of infection. If, however, blind therapy is necessitated by the clinical situation, we would treat broadly to cover both gram-positive and gram-negative organisms that take advantage of neutrophil defects (Table 12–1); specific recommendations are discussed in the section on treatment.

Common causes of obscure fevers in patients with lymphatic neoplasms, especially those with defects in cellular immunity, are disseminated tuberculosis and cytomegalovirus. One must have a high index of suspicion for disseminated tuberculosis since the typical military pulmonary lesions may be absent, and the clinical presentation may even mimic acute or chronic leukemia. The diagnosis is particularly crucial to make since excellent antituberculous therapy is available.

All patients who have received multiple blood transfusions are at risk for contracting malaria (as well as the more commonly considered viral hepatitis), and thick and thin smears to detect *Plasmodium* organisms should be done in these patients with unexplained fever. In areas where *Babesia* species are endemic they should be looked for on smear as well.

Organisms Frequently Responsible for Infection

We have found that it is most convenient to consider the various infections in terms of clinical presentations, and the following discussion is presented in this manner.

Skin, Bone, and Joint Infections

Skin lesions present a readily accessible site for aspiration and biopsy, enabling a presumptive (by smear) and then specific (by culture and/or histopathology) diagnosis to be quickly and easily

TABLE 12–1 Immune Defects and Infecting Organisms to Be Anticipated in Patients with Neoplastic Disease

Immune Defect and Examples	Bacteria	Fungi	Parasites	Viruses
Interrupted integument				
Bladder catheters	*Streptococcus pyogenes*	*Candida* spp.		
Intravenous needles	*Staphylococcus aureus*			
Catheters	Enteric bacilli			
Finger or bone marrow puncture	*Staphylococcus epidermidis*			
Chemotherapy-induced gastrointestinal ulcers	*Corynebacterium* spp.			
Infusion infections				
Blood products	*Salmonella* spp.	*Candida* spp.	*Plasmodium* spp.	Hepatitis B
Intravenous fluids	*Enterobacter agglomerans*		*Toxoplasma gondii*	Cytomegalovirus
	Enterobacter cloacae			Epstein-Barr virus
				Hepatitis non-A & B
Surgical procedures	*Streptococcus pyogenes*	*Candida* spp.		
Tracheostomy	*Staphylococcus aureus*			
Respiratory assistance	Enteric bacilli			
Endoscopies	*Bacteroides fragilis*			
Gastrointestinal or gynecologic surgery				
Spleen				
Staging laporatomy for Hodgkin's disease	*Neisseria meningiditis*		*Babesia* spp.	
	Hemophilus influenzae		*Plasmodium* spp.	
	Streptococcus pneumoniae			
Neutrophil				
Acute leukemia	*Streptococcus pyogenes*	*Candida* spp. (disseminated)		
Chemotherapy	*Staphylococcus aureus*	*Aspergillus* spp.		
	Enteric bacilli	Mucoraceae		
		Trichosporon cutaneum		
T lymphocyte mononuclear phagocyte				
Hodgkin's disease	*Listeria monocytogenes*	*Cryptococcus neoformans*	*Pneumocystis carinii*	Measles
Chemotherapy	*Salmonella* spp.	*Candida* spp. (local)	*Toxoplasma gondii*	Varicella zoster
	Nocardia asteroides	*Histoplasma capsulatum*	*Strongyloides stercoralis*	Cytomegalovirus
	Mycobacterium spp.	*Coccidioides immitis*		
	Legionella spp.	*Blastomyces dermatiditis*		Adenovirus
Gammaglobulin				
Multiple myeloma	*Streptococcus pneumonia*		*Pneumocystis carinii*	
Chronic lymphocytic leukemia	*Hemophilus influenzae*			
Chemotherapy	Enteric bacilli			

made. This should be done promptly because seldom is it possible in patients with lymphatic neoplasm to predict the infecting organism by clinical appearance alone.

Patients with neutropenia are predisposed to skin infection with *Staphylococcus aureus* and *Streptococcus pyogenes,* which act as primary invaders of the intact or disrupted integument. These infections can be well controlled with penicillin (*Strep. pyogenes*) or a semisynthetic penicillin (*Staph. aureus*). Enteric bacilli, including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter* species, and *Aeromonas hydrophilia,* also have a predilection for the neutropenic patient, but generally cause skin infection secondary to bacteremic spread or entry through a disruption in the skin. *Pseudomonas aeruginosa,* which is a highly resistant organism, often multiplies in the stool of patients receiving multiple antibiotics. In this situation, a cellulitis due to *Ps. aeruginosa* may commonly develop in the area of an anal fissure, spread rapidly, and subsequently lead to metastatic skin lesions by traveling through the blood stream. These metastatic lesions appear as the classical "ecthyma gangrenosum" lesion: papular lesions with a black central area of necrosis. In fact, this lesion may be due to any of the other enteric bacilli, as well as to fungi such as *Aspergillus* species, Mucoraceae, *Candida* species,[21] and *Trichosporon cutaneum.* Subcutaneous emphysema associated with cellulitis, particularly on the trunk, flanks, and thighs, may indicate a *Clostridium* species infection, but may also be caused by a gas-producing mixed gram-positive and gram-negative infection (for example, microaerophilic streptococci, *E. coli,* and *Bacteroides fragilis*).

Candida tropicalis[48] may produce a particularly fulminant syndrome in neutropenic patients, typified by severe muscle pain and tenderness due to myositis and macular–papular skin lesions which develop dark centers. Disseminated *Candida* skin lesions may be accompanied by metastatic retinal lesions in the form of large, cottonball exudates, and this is an important sign to check for in evaluating the neutropenic patient with disseminated skin lesions. Disseminated skin lesions due to fungi require systemic treatment with amphotericin B. Those caused by enteric bacilli are treated with an aminoglycoside and carbenicillin (for *Ps. aeruginosa*) or cephalosporin (per sensitivity). Finally, infection with *Staph. epidermidis*[75] and *Corynebacterium* species CDC-JK[66,78] have become more prevalent with the use of prolonged intravenous catheters, particularly in the setting of prolonged and profound neutropenia. These two organisms may be widely antibiotic resistant and have been consistently sensitive only to vancomycin. In some hospitals in the United States, appreciable resistance to methicillin has appeared among *Staph. aureus* isolates and these require vancomycin.

Patients with impairment of cellular immunity are vulnerable to skin infection by a different group of organisms. Although only aspiration or biopsy will reliably distinguish among *Mycobacterium* species, *Nocardia, Cryptococcus, Histoplasma, Coccidioides,* and *Toxoplasma,* generally varicella or herpes zoster will be clinically obvious. The latter two are caused by the same virus: varicella (chicken pox) represents an initial infection and herpes zoster (shingles) represents recrudence of a latent infection. Either may cause severe, progressive disease in patients with lymphomas, although patients with advanced Hodgkin's disease are particularly vulnerable to dissemination, and this risk may be increased by prior splenectomy.[53] Clinical trials are in progress studying the effect of new antivirals[13] in the treatment of herpes zoster, with some promise from agents such as arabinosyl adenine or acycloguanine. Lesions of herpes zoster are occasionally confused with herpes simplex, but a dermatomal distribution (when present) and a culture of the base of the vesicular lesion should discriminate, whereas smears cannot.

Bone, joint, and muscle lesions are caused by hematogenous dissemination of the same organisms causing superficial skin lesions. Aspiration or biopsy is necessary to make a specific diagnosis; this is particularly important since these deep infections require 4–6 weeks of treatment to ensure eradication.

Stomatopharyngitis

Stomatopharyngitis is a common occurrence in patients with lymphatic neoplasms receiving intensive chemotherapy; and it is usually due (at least in part) to the antineoplastic drugs, which selectively attack rapidly regenerating tissues. Often, however, there is a concomitant infectious contribution to the stomatitis, presumably due to mixed mouth flora and sometimes specifically due to *Candida albicans* or herpes simplex; any of these or-

ganisms can also extend downward to produce an esophagitis as well. It is impossible clinically to distinguish between purely drug toxic and infectious stomatitis, but use of smears (to look for *Candida pseudohyphae*), cytology (looking for herpes simplex inclusions), and culture will suggest the diagnosis. Treatment of herpes simplex stomatitis or esophagitis is limited to symptomatic relief; *Candida* stomatitis can usually be treated with topical nystatin alone, but esophagitis may require initiation of low-dose (0.3 mg/kg/day) amphotericin B, or Ketoconazole, 200 mg, p.o. twice a day.

Septic-appearing neutropenic patients with pharyngitis must be treated as medical emergencies, with the presumptive diagnosis of an enteric gram-negative bacillary infection as the cause of the pharyngitis. These can be rapidly lethal and must be treated intravenously with a regimen (see section on treatment) that will cover *Ps. aeruginosa, K. pneumoniae,* and *E. coli,* as well as *Staph. aureus* and *Strep. pyogenes.* Patients with lymphoma after prolonged leukopenia and antibiotic therapy are susceptible to rhinocerebral mucormycosis. If a black crusted lesion is visible in the posterior pharynx or nasopharynx, it should be scraped and examined by potassium hydroxide wet mount (to look for the broad, ribbonlike, nonseptate hyphae of *Mucor*)[49] as well as by gram stain (*Ps. aeruginosa* may produce an identical black necrotic lesion). At times, however, the diagnosis may be suggested only by clouding of a maxillary sinus, and in these cases, a more aggressive surgical approach for diagnosis (methenamine silver stain will enable visualization of the broad nonseptate hyphae in biopsy material) and treatment must be undertaken. Amphotericin B should be started in cases of documented mucormycosis or even where it is strongly suspected; and antipseudomonal treatment should be given as well until the microbial diagnosis is established.

Patients with impaired cellular immunity are susceptible to particularly severe local herpes simplex lesions which may involve the whole mouth; but even in these patients dissemination is extremely rare. *Candida* lesions in this group usually respond to intensive local therapy and (when possible) decrease or cessation of the predisposing factor (for example, corticosteroid therapy). Mouth ulcers in lymphoma patients should also suggest the possibility of histoplasmosis if the patient has visited or lived in endemic areas, and these lesions should be biopsied as well as smeared and cultured.

Central Nervous System Infections

The causes of meningitis in patients with lymphatic neoplasm differ markedly from those in the normal population, in whom *Strep. pneumoniae, Neisseria meningitidis,* and (in children) *Hemophilus influenzae* are the primary considerations. These organisms may occur in lymphoma patients, especially in those patients who have undergone splenectomy or have gamma globulin defects, in whom particularly severe and recurrent disease may occur, almost always due to *Strep. pneumoniae.* With other causes of meningitis, however, signs in the lymphoma patient may be quite subtle: minimal personality changes, low-grade (or even absent) fever, or mild (or absent) meningismus. Early diagnosis requires a high index of suspicion and readiness to perform a lumbar puncture when signs are only minimal. (Platelet transfusion should be given prior to the spinal tap if the platelet count is less than 50,000/mm^3). The type of infecting agent is usually predictable in suspected central nervous system (CNS) infections by knowing the type of immune defect; the possibility of neoplastic involvement must also be considered, particularly in patients with leukemic transformation of the lymphoma.

Neutropenic patients are vulnerable to meningitis caused by aerobic gram-negative rods: *E. coli, K. pneumoniae,* and *Ps. aeruginosa,* which almost always cause a fulminant infection. Although neutrophils are absent on gram stain, the organisms are readily visualized and grow rapidly in culture. We feel that optimal treatment includes a carbenicillin-type drug and an aminoglycoside, the latter given intravenously as well as directly into the cerebrospinal fluid (CSF)—intraventricularly through an Ommaya reservoir is superior to intrathecal administration[31]—although some researchers have reported success in treating gram-negative bacillary meningitis with some of the third-generation cephalosporins such as moxalactam.[39] Brain abscess in these patients may occasionally be caused by enteric bacilli, but should suggest also infection with *Aspergillus* spp. or Mucoraceae. In cases of aspergillus brain abscess there is frequently an accompanying lung lesion, and conversion from a negative to a positive *Aspergillus* antibody test may be demonstrable. Patients with mucormycosis may have the rhinocerebral form, in which case diagnosis may be made by scraping the nasopharynx. In most cases of both aspergillosis and mucor-

mycosis, however, diagnosis requires brain biopsy, since the CSF shows a nondiagnostic response and the organism is rarely seen in the CSF.[51] *Candida* species may cause CNS infection secondary to fungemia, producing either a low-grade meningitis or brain abscess(es). The organisms are seldom isolated from the CSF, and diagnosis is seldom made antemortem.

Patients with T cell mononuclear phagocyte defects are susceptible to a wide range of pathogens capable of causing CNS infection; however, *Listeria monocytogenes* and *Cryptococcus neoformans* are the two most often seen.[12] *Listeria* meningitis may present with subtle CNS signs, or with fever and bacteremia alone.[43,44,54] On gram stain of the CSF, this gram-positive rod may not be seen, or it may be mistaken for gram-positive diplococci, or gram-negative rods if overdecolorized. Occasionally, laboratories may identify *L. monocytogenes* as a "contaminating diphtheroid," but *Listeria* organisms are readily distinguished from diptheroids by virtue of their motility and their ability to produce hemolysis on blood agar. A critical point in the management of immunosuppressed patients is that the decision regarding whether or not an isolate represents contamination must be made by the clinician and not by the laboratory; and in order for the physician to do this intelligently, he must be supplied with a full identification of organisms isolated. Treatment of *Listeria* meningitis requires prolonged administration (4 weeks) of ampicillin or penicillin to ensure eradication of this persistent, intracellular pathogen. *Legionella pneumophila* also takes advantage of cellular immune defects, but its frequency as a cause of meningoencephalitis remains to be determined.

Cryptococcal meningitis may also present with subtle signs, and the organism may be seen on gram stain or India ink preparations (both should be done) of CSF sediment. Even in the absence of visualization of the organism, the diagnosis may be made rapidly by detection of specific capsular antigen, usually by using a Latex agglutination test. Although this may be the only means of making the diagnosis, in our hands in every case but one, whenever cryptococcal antigen was detected, procurement of additional CSF allowed isolation of the organism.[33] Detection of antigen provides not only an excellent diagnostic tool, but also a parameter by which to gauge duration of therapy: we generally favor continuation of amphotericin B for about 1 month after the disappearance of detectable antigen. Other advances in the treatment of this disease include administration of amphotericin B intraventricularly via an Ommaya reservoir and use of 5′-fluorocytosine.[46] Transfer factor and rifampin have been added to amphotericin B, but there is insufficient evidence to evaluate their efficacy.

Other less common causes of meningitis in lymphoma patients include *Histoplasma capsulatum* and *Coccidioides immitis* (with history of residence in or travel to endemic areas) occurring in the setting of disseminated disease. CNS tuberculosis has been surprisingly rare in such patients.

Dissemination of primary or latent toxoplasmosis frequently involves the CNS in patients with lymphoma, where it causes brain abscess or encephalitis.[11,57] Serology may allow a presumptive diagnosis when a fourfold or greater rise in titer is detected, but there are both false-positive and false-negative results. Early diagnosis is imperative because mortality is high even in treated cases; this not uncommonly requires brain biopsy after demonstration of the focal mass on computerized axial tomographic scan. Treatment is with sulfonamide and pyrimethamine; duration of treatment is somewhat empiric, but is at least 6 weeks. The presence of a brain abscess should also raise the suspicion of nocardiosis, particularly in the setting of associated pneumonia and skin lesions. Brain biopsy may be required for diagnosis (if there are no other clinically suspicious areas to biopsy), and may be important for therapeutic drainage. Treatment is with a sulfonamide with or without trimethoprim, and again, duration of treatment depends on rapidity of response, but should be at least 2 months. Other causes of encephalitis include varicella zoster (usually with obvious cranial nerve zoster) or herpes simplex (usually in the absence of oral lesions). Diagnosis of the latter usually requires brain biopsy and treatment is with adenine arabinoside or acycloguanine.

Pulmonary Infections

Pneumonias are a frequent infectious complication in patients with lymphatic neoplasms and may well be lethal in this setting. Any of the organisms to be discussed may present as acute diffuse pneumonias, although this presentation is most typical of certain organisms that take advantage of cellular immune defects. Diagnosis of pneumonia is more

difficult in immunocompromised patients because the usual rapid means of making a presumptive diagnosis, that is, the sputum exam, is often unavailable. The virtual absence of polymorphonuclear leukocytes in neutropenic patients retards the production of an exudate, which results not only in slowing the development of physical and x-ray signs of pneumonia, but also reduces the amount of sputum produced. Furthermore, if a specimen is available for examination, it is often difficult to be sure that it represents lower respiratory secretions rather than saliva; in the absence of polymorphonuclear leukocytes on smear, one must rely on the absence of normal mouth flora and predominance of a likely pathogenic organism to assess the reliability of the specimen. In patients with cellular immune defects there is a predisposition to diffuse alveolar and interstitial pneumonias; in these cases, cough is usually nonproductive and sputum is unavailable.

In all types of immunocompromised patients specific diagnosis is critical, and if less invasive tests, such as aspergillar antibodies,[23,62] cryptococcal antigen,[22] bronchoalveolar lavage, and direct fluorescent antibody,[29] fail to yield a diagnosis, open lung biopsy has been increasingly performed. This is preferable to needle biopsy or aspiration insofar as it reduces sampling error, enables better control of potential bleeding and may reveal two or more treatable causes rather than one. Subsegmental pulmonary lavage, which samples the alveolar space and is performed via flexible bronchoscopy, appears promising and may reduce the number of cases requiring open lung biopsy.[35]

Neutropenic patients presenting with pneumonia are most often infected with *Staph. aureus* or enteric bacilli, and pending definitive diagnosis should be treated for these organisms. Mortality due to pneumonia associated with bacteremia may be as high as 76% in these patients,[64] and even higher when *K. pneumoniae* or *Ps. aeruginosa* are responsible. Most often these pneumonias represent primary infection; but in certain instances, they may be the result of hematogenous seeding, for example, with *Candida* species.[45] These rarely cause pneumonia, but when present, hematogenously seeded *Candida* pneumonia will usually not be accompanied by organisms in the sputum. *Cornyebacterium* CDC-JK, a resistant *Cornyebacterium* species found in certain cancer centers to be responsible for invasive disease in long-term neutropenic patients on multiple antimicrobials, may also spread to the lungs and is frequently lethal.

Pseudomonas aeruginosa pneumonia as well may develop secondary to bacteremia, usually from a gastrointestinal source, in which case it will appear as a rapidly developing peripheral, wedge-shaped infiltrate indistinguishable from *Aspergillus*[51] or Mucorales pneumonia.[41,50] The latter two organisms must be particularly suspected in patients who have been in the hospital receiving broad-spectrum antibiotics and suddenly develop a pneumonia. Sputums in this situation are unreliable since both false-negative and false-positive results may occur; although repeatedly positive sputa in a febrile patient not previously colonized should prompt specific therapy. Early diagnosis and treatment of aspergillosis may be possible by detection of new or rising precipitins to *Aspergillus* in prospectively collected sera; early treatment permits response rates of more than one-third of cases treated.[4,23,62] Experimental tests for antigens—in either blood or bronchial washings—appear promising.[1] Unfortunately, no such serologic test is available for mucormycosis.

Pneumonia in patients with defects in cellular immunity may present in a variety of ways. Infection with *Nocardia asteroides*[38,76] generally presents with progressive respiratory symptoms and necrotizing abscesses in the lung, although the chest roentgenogram may vary from diffuse infiltrative disease to solitary nodules with or without cavitation.[27] Repeated sputum specimens usually yield the diagnosis either on smear or in culture, provided the culture plates are kept long enough (21 days, since this is a slow-growing organism). It is critical to treat this infection promptly because of the organism's predilection to involve the CNS.

Tuberculosis[32] in these patients may mimic a fulminant bacterial pneumonia and initial treatment for tuberculous pneumonia should utilize a three-drug regimen (such as isoniazid–streptomycin–ethambutol or isoniazid–streptomycin–rifampin). Preventive recommendations include purified protein derivative (PPD) testing of patients at the time of initial diagnosis and use of an additional battery of skin tests to assess energy in those patients with a negative PPD skin test. In patients with a positive tuberculin test, or in those who are anergic and for whom heavy immunosuppressive treatment (especially steroids) is contemplated, prophylactic isoniazid should be given for at least 1 year.

In these patients, *Cryptococcus neoformans* may be responsible for an acute interstitial pneumonia or for nodular lesions, but presence of organisms in the sputum does not necessarily correlate with invasive disease. A serum positive for cryptococcal antigen in the setting of pulmonary lesions, however, should be considered sufficient indication to treat with amphotericin B. In patients with a history or residence in or travel to endemic areas, primary or recrudescent *Histoplasma capsulatum*[34] and *Coccidioides immitis*[15] infection must be considered in the differential diagnosis of pulmonary lesions. Neither appears to occur with increased frequency in immunocompromised hosts, but the severity of the disease, particularly as reflected in rates of dissemination, is increased in these patients. Skin testing is not helpful in either of these because the combined factors of high prevalence of positivity in endemic areas and impairment of delayed type hypersensitivity reactions in this patient population make positive or negative results meaningless in the individual patient. Serology may be useful in the diagnosis of either, but false-negative results are common. In either case, however, biopsy specimens stained with Giemsa or methenamine silver may permit diagnosis; in cases of disseminated histoplasmosis, bone marrow tissue has been particularly valuable in visualizing the organisms.

Foremost among the organisms causing acute diffuse pneumonias in patients with cellular immune defects is *Pneumocystis carinii,* a parasite that appears to be a widespread latent agent. Diagnosis may occasionally be made by Gram–Weigert stain of sputum or tracheal aspiration, but usually requires open lung biopsy,[60,71] or the more recently introduced technique of subsegmental pulmonary lavage.[35] Since trimethoprim–sulfamethoxazole has become recognized as an effective agent in treating this infection,[61] many choose to defer open lung biopsy in suspected cases pending a therapeutic trial with that drug; if there is no response after 3–5 days and no diagnosis by other means, then open lung biopsy should be performed to search for other treatable conditions. Based on the clinical observation that the appearance of *P. carinii* pneumonia frequently coincides with periods of tapering or discontinuation of steroid therapy, many centers have incorporated prophylactic trimethoprim–sulfamethoxazole into their treatment protocols for children with acute lymphoblastic leukemia, and have noted a gratifying reduction in the incidence of *P. carinii* infections.[28] Patients with suspected or proven *P. carinii* pneumonia should be kept in isolation because of the possibility of patient-to-patient spread suggested by a report of a cluster of cases.[63] Cytomegalovirus and, less commonly, herpes simplex may also be responsible for acute diffuse pneumonias and are usually seen in combination with other infectious pneumonias in patients with far advanced lymphomas.[58,59] A rise in antibody titer or isolation of virus from sputum, blood, or lung biopsy does not necessarily indicate pneumonia as due to the virus,[3] which requires demonstration of typical histopathologic changes. There is no known effective treatment for pneumonia due to either viral infection, but a variety of clinical trials with potential antiviral agents are currently being conducted.

Gastrointestinal Infections

Intra-abdominal and gastrointestinal infections in lymphoma patients are similar to those in the general population, but often present with only minor physical signs even in the presence of an acute abdominal catastrophe. This is particularly true in patients receiving corticosteroids, but applies as well to neutropenic patients.

Neutropenia in association with thrombocytopenia and chemotherapy-induced gastrointestinal ulcers would appear to predispose the patient to the development of sepsis with enteric organisms (*E. coli, K. pneumoniae, Ps. aeruginosa, Proteus* species, *Enterobacter* species, *Aeromonas hydrophila*), which enter the bloodstream through breaks in the intestinal mucosa. In fact, autopsy studies in such patients have demonstrated ulcers in the gastrointestinal tract which contain gram-negative rods or *Candida* species and have suggested that the gastrointestinal tract is the most common source of bacteremia of undocumented origin. *Clostridium* species (usually *Cl. perfringens* or *Cl. septicum*) may invade the gastrointestinal tract of neutropenic patients or of lymphoma patients with bulky, necrotic tumor involving the bowel. The latter circumstance provides the clostridia access to an anaerobic environment of poorly perfused tissue and predisposes to the syndrome of clostridial sepsis.[74] This may be indistinguishable from that due to other organisms, or may present in one of two distinct forms. The classical syndrome of massive hemolysis, tachycardia out of proportion to fever, and alertness despite hypoten-

sion has been the exception. More commonly, there is impending or intermittent obstruction followed by cellulitis of flanks, trunks, even thighs and axillae, which within hours becomes ecchymotic and then crepitant. Aspiration of the border of the cellulitis will discriminate clostridial infection from cellulitis due to mixed aerobic and anaerobic bowel flora by revealing cherry-red fluid that is relatively acellular but full of large gram-positive rods. The gram-positive rods may even be seen in the peripheral blood smear. Prompt treatment with high-dose penicillin in association with relief of obstruction and removal of necrotic tissue has increased the survival rate to approximately 50% in a disease which is otherwise rapidly fatal within 12–24 hours.

Candida species, particularly *C. albicans* and *C. tropicalis,* may invade throughout the gastrointestinal system, leading to clinical sepsis without demonstrable source. Difficulties in diagnosing invasive candidiasis infection arise from the following considerations: isolation of *Candida* organisms from most body sites generally reflects colonization; isolation from blood culture in cases of invasive disease is accomplished usually in less than 50% of patients with ultimately proven dissemination, and often with a delay of 3–7 days; serologic tests for antibody are unreliable, revealing both false-positive and false-negative results;[20] and tests for circulating antigen,[69] cell constituents,[52] and metabolites[36] are being evaluated but require confirmation regarding specificity and utility in making early diagnoses. The question of when to initiate amphotericin B therapy in the septic patient without established etiology is discussed in the section on clinical sepsis.

Less problematic is the approach to *Candida* esophagitis, a common problem in neutropenic patients receiving antibiotics. In the appropriate setting, the symptoms of food sticking in the midesophagus on swallowing should suggest this diagnosis and prompt institution of large doses of nystatin (30–40 million units/day, usually following a "swich and swallow" regimen). If this is ineffective within 24–48 hours, we proceed to using low-dose amphotericin B (0.3 mg/kg/day). Clotrimazole[25] or ketoconazole have also recently been reported effective in refractory *Candida* esophagitis. We have not found esophagrams to be helpful (they may be negative, or reflect herpes simplex or bacterial esophagitis) and esophagoscopy may result in sepsis[26] or perforation.

Defects in cellular immunity predispose to particularly severe syndromes involving *Salmonella* species[73] and *Strongyloides stercoralis.*[7,68] Non-typhi serotypes of *Salmonella,* which almost exclusively cause enteritis in normal hosts, usually cause a septic syndrome in patients with leukemia and lymphoma and may be responsible for a focal infection as well, frequently with localization in neoplastic tissue. When the disease is limited to a gastroenteritis in the immunocompromised host, it is generally severe and should be treated to prevent the development of sepsis. The antibiotics consistently effective in vivo against salmonellae have been ampicillin, trimethoprim–sulfamethoxazole, and chloramphenicol.

Strongyloides stercoralis is capable of producing a severe hyperinfection syndrome, particularly in patients receiving steroids, at a time remote from the initial infection, owing to its ability to persist, autoinfect, and multiply within the human host. In addition to abdominal pain with ileus and distention, the patient with disseminated disease may manifest fever, polymicrobic sepsis, pulmonary symptoms and infiltrates, and neurologic dysfunction due to mixed microbial meningitis; all are caused by either the carriage of bacteria along with larvae, or their penetration into the circulation through *Strongyloides*-induced gut ulceration.[47] At this point, large numbers of characteristic larvae may be identified in stool, sputum, or CSF, but the disease may rapidly progress despite initiation of thiabendazole therapy. Hence, it is preferable to identify the presence of *Strongyloides* infection at a time when symptoms are absent or minimal. Unexplained eosinophilia and a history of birth in or travel to endemic areas should suggest the possibility, which is pursued by examination of stool and duodenal samples, the latter collected using the Enterotest.[5] Some investigators have suggested presumptively treating with thiabendazole for 2 days when dealing with patients with the appropriate residence or travel history, prior to initiation of steroid treatment. Cytomegalovirus may also cause enteritis and ulcers of the gastrointestinal tract; diagnosis is accomplished only by biopsy and treatment remains experimental.

Genitourinary Infections

Genitourinary infections in immunocompromised hosts resemble those in the general population,

although a few distinguishing points need to be considered. In immunocompromised patients, urinary tract infections occur more frequently and *Candida* isolates are more likely to represent true infection with potential to disseminate. This reflects not only the underlying disease or chemotherapy, but, just as importantly, factors such as manipulative procedures, indwelling catheters, prolonged antibiotic treatment with suppression of normal flora, and urinary tract obstruction due to intra-abdominal or pelvic neoplasms.

The usual enteric bacilli and enterococci are seen most often as urinary pathogens, but *Serratia marcescens* and *Staph. epidermidis* may appear in clusters, particularly in intensive care units, and *Ps. aeruginosa* presents the greatest therapeutic challenge as more sensitive organisms are eradicated by less toxic antibiotics. Even with *Ps. aeruginosa,* however, the likelihood of surviving a bacteremia is much higher when the source is the urinary tract rather than the lungs or gastrointestinal tract[64] because of the relative ease of ensuring drainage. *Pseudomonas aeruginosa* infection may also originate in the vagina of neutropenic patients, where it presents as rapidly progressing cellulitis with central necrosis. Appropriate antibiotics and debridement usually only contain the infection until the leukocyte count returns, at which point the necrotic tissue will slough as the infection is eliminated.

In the neutropenic patient with suspected urinary tract infection, pyuria is absent, but a gram stain can rapidly supply a presumptive diagnosis. Visualization of bacteria on a fresh centrifuged urine sample reflects a colony count of approximately 1×110^4/ml, and this is considered significant in the neutropenic patient. The same sort of guideline cannot be given for *Candida* species in the urine, although large numbers of organisms in a neutropenic patient should be regarded as significant. Renal failure may develop with advanced Candida infection, either due to renal replacement or in association with obstruction due to fungus balls. Patients with indwelling Foley catheters are particularly prone to this, and obstruction can occur at either the level of the ureterovesical junction or in the renal tubules. If the blockage is low, bladder irrigation with 50 mg amphotericin B per liter 5% dextrose in water may be effective; otherwise, low-dose (20–30 mg/day) systemic amphotericin B (although a renal toxic drug) can be lifesaving.

Therapeutic Approach in Clinical Sepsis

If time permits and the source of infection can be identified so that an organism can be isolated, therapy can be determined by the antimicrobial sensitivity pattern of the offending organism(s). The antimicrobial agents of choice for organisms that commonly infect patients with lymphatic neoplasms are listed in Table 12–2; the usual doses administered are given in Table 12–3. Certain of the recommendations may vary among hospitals depending on local resistance patterns.

In the many instances when specific microbial diagnosis is not yet available or cannot be made, a presumptive diagnosis must be made to permit a rational decision regarding therapeutic intervention. A presumptive microbial diagnosis is made on the basis of the underlying disease and therapy, prior or concurrent antimicrobial therapy, previous infection with latent organisms, environment (including presence of catheters and prosthetic devices, as well as community versus in-hospital acquisition of infection), and, most importantly, microscopic examination (by gram stain, potassium hydroxide preparation, AFB stain) of any relevant body fluids or exudates.

It is frequently necessary to begin treatment of suspected infection with no clue as to the causative pathogen other than that suggested by the underlying immune defect. This is particularly true in neutropenic and splenectomized patients, in whom fulminant sepsis can be lethal within 12–24 hours. It is less often the case in patients with cellular immune defects, in which case time is usually available to investigate possible causes; although it should be remembered that in these patients tuberculosis and strongyloidiasis can be fulminant infections.

Drug Regimens

Suggested empiric regimens for initial treatment of clinical sepsis are outlined in Table 12–4. Neutropenic patients generally receive a regimen including an antipseudomonal penicillin and an aminoglycoside. A primary antistaphylococcal drug (semisynthetic penicillin, cephalosporin, or vancomycin) should be used in strongly suspected or documented *Staph. aureus* infection, but pending positive cultures for *Staph. aureus,* an aminoglyco-

TABLE 12–2 Antimicrobial Agents of Choice for Specific Microorganisms

Organism	Drug of Choice	Alternatives
Gram-positive cocci		
Streptococcus pyogenes or *pneumoniae*	Penicillin	Erythromycin, cephalosporins
Anaerobic stroptococci	Penicillin	Tetracyclines, erythromycin, vancomycin, clindamycin
Staphylococcus aureus	Penicillinase (resist penicillin)	Vancomycin, cephalosporin, clindamycin
Staphylococcus epidermidis	Vancomycin	
Gram-positive bacilli		
Clostridium perfringens	Penicillin	Tetracyclines, choramphenicol, cefoxitin, clindamycin, cephalosporins
Listeria monocytosis	Ampicillin, penicillin (+/− Gent)?	Erythromycin, chloramphenicol, tetracyclines
Cornyebacterium DCD-JK	Vancomycin	
Gram-negative bacilli		
Hemophilus influenzae	Chloramphenicol, ampicillin (if β-lactamase negative)	Trimethoprim–sulfamethoxazole, cefamandole
Legionella pneumophila	Erythromycin +/− Rifampin	Tetracyclines
Salmonella (non-typi)	Ampicillin, amoxicillin	Chloramphenicol, trimethoprim–sulfamethoxazole
Escherichia coli	Aminoglycoside	Ampicillin, cephalosporin, chloramphenicol, trimethoprim–sulfamethoxazole
Klebsiella pneumoniae	Aminoglycoside	Cephalosporin, chloramphenicol, trimethoprim–sulfamethoxazole
Proteus mirabilis	Ampicillin	Cephalosporin, Aminoglycoside, chloramphenicol, trimethoprim–sulfamethoxazole
Pseudomonas aeruginosn	Aminoglycoside with carbinicillin	Moxolactam, colistin
Acinetobacter	Aminoglycoside	Minocycline, TMP-sulfa
Bacteroides	Clindamycin (penicillin for oropharyngeal infection)	Chloramphenicol, cefoxitin, metronidazole
Enterobacter	Aminoglycoside	Chloramphenicol, TMP-sulfa, cefamandole, carbenicillin
Mycobacteria		
Mycobacterium tuberculosis	Isoniazid, ethambutal, rifampin	Isoniazid plus one or two of the following: streptomycin, *p*-aminosalicylic acid, pyrazinamide, cycloserine, ethionamide, kanamycin, capreomycin
Nocardia	Sulfonamide, trimethoprim–sulfamethoxazole	Cycloserine, minocycline, ampicillin erythromycin
Fungi		
Cryptococcus	Amphotericin B[5] with 5′-fluorocytosine	Miconazole
Candida species	Amphotericin B	5′-Fluorocytosine, ketoconazole
Aspergillus	Amphotericin B	
Coccidioides	Amphotericin B	Ketoconazole
Histoplasma	Amphotericin B	
Protoza		
Pneumocystis carinii	Trimethoprim–sulfamethoxazole	Pentamidine, pyrimethamine–sulfonaminde
Toxoplasmosis	Pyrimethamine–sulfonamide	Pyrvinium pamoate
Strongyloides	Thiabendazole	

TABLE 12–2 (con't) Antimicrobial Agents of Choice for Specific Microorganisms

Organism	Drug of Choice	Alternatives
Viruses		
Varicella zoster	Adenine arabinoside	Acycloguanine
Herpes simplex	Adenine arabinoside	Acycloguanine
Cytomegalovirus	Adenine arabinoside	Acycloguanine

side may be sufficiently effective to contain the infection. A cephalosporin is often added in hospitals where *K. pneumoniae* is a common infecting organism for the theoretical possibility of enhanced effectiveness. Increased renal toxicity with a ceophalosporin–aminoglycoside combination remains to be proved for the newer cephalosporins and is even questionable with cephalothin. The role of new, investigational third-generation cephalosporins is not clear. They may provide an alternative to carbenicillin/ticarcillin in patients who are penicillin allergic. If used, we favor their use in conjunction with an aminoglycoside rather than as sole therapy for patients who are neutropenic.

In patients with lymphomas involving the gas-

TABLE 12–3 Usual Dosages of Antimicrobial Agents

Drug	Usual Daily Dose (mg/kg) (in grams/day)	Dosing Interval (hours)[a]
Amikacin	15	q12 or q8
Ampicillin	200 (12)[b]	q6 or q4
Amphotericin	1	Once daily
Carbenicillin	400–600 (30–40)	q4
Chloramphenicol	50–100 (4)	q6
Clindamycin	30–40 (2–3)	q6
Cefamandole	100–200 (12)	q6
Cefazolin	50–100 (6)	q6 or q4
Cefoxitin	100–200 (12)	q6 or q4
Cycloserine	15 (0.75–1)	q6 or 12
Erythromycin	15–50 (4)	q6
Ethambutol	15	Once daily
Ethionamide	10–15 (0.5–1)	q8–24
5'-Fluorocytosine	150	q6
Gentamicin/tobramycin	5	q6
Isoniazid	3–5 (0.3)	Once daily
Moxolactam	50–200 (4–12)	q8
Oxaxillin/nafcillin	100–200 (12)	q6 or q4
PAS	200 (10–12)	q8–24
Penicillin G	20,000,000 units/day	q6 or q4
Pyrimethamine	(25 mg) after 100 mg load	
Pentamidine	4	Once daily (14 days)
Pyrazinamide	25 (maximum 2.5)	Once daily
Sulfonamide	Varies with drug	
Rifampin	9 (0.6)	Once daily
Streptomycin (for tuberculosis)	15 (1)	q12 or q24
Tetracycline	10–20 (1)	q6
Thiabendazole	50	Twice daily (2 days)
Ticarcillin	250–300	q4
Trimethoprim–sulfamethoxazole[c]	1 tablet/4 kg (for *Pneumocystis*) 2 tablets bid (for other)	q6 or q8
Vancomycin	(4)	q6

[a] Shorter interval recommended for neutropenic patients.
[b] Dose in g/day is given in parentheses.
[b] Each tablet contains 80 mg trimethoprim and 400 mg sulfamethoxazole.

TABLE 12–4 Suggested Regimens for Empiric Treatment of Sepsis

Drug	Dosage
Oxacillin[a b]	50.0 mg/kg i.v. q6 hours
Cefazolin[a]	50.0 mg/kg i.v. q6 hours
Gentamicin[a b c]	1.25 mg/kg i.v. q6 hours
Amikacin[c]	5.0 mg/kg i.v. q8 hours
Tobramycin[c]	1.25 mg/kg i.v. q6 hours
Carbenicillin[b d]	85.0 mg/kg i.v. q4 hours

[a] For presumed sepsis.
[b] When neutropenia is present (oxacillin or cefazolin can be omitted in the absence of pneumonia or skin lesions).
[c] According to susceptibility patterns, gentamicin, amikacin, or tobramycin can be used in a particular hospital. *** Third-generation cephalosporin
[d] Ticarcillin.

trointestinal tract or gynecologic tract, coverage for *Bacteroides fragilis* or *Clostridium* species should be considered, although carbenicillin or ticarcillin may cover these organisms. If documented, however, *B. fragilis* should be treated with chloramphenicol, clindamycin, metronidazole or cefoxitin, and *Clostridium* species are optimally treated with large doses of penicillin G. Splenectomized patients need presumptive treatment for *Strep. pneumoniae, Neisseria meningitidis,* and *Hemophilus influenzae;* penicillin or ampicillin in high doses should be started promptly after cultures are drawn, and if the situation is acute, chloramphenicol should be used for possible ampicillin-resistant *H. influenzae.*

Considerably more difficult than the decision regarding initial treatment in neutropenic patients without a documented source of infection are the following questions: How long should antibiotics be continued? When is a change in antibiotics indicated? When should treatment of possible fungal superinfection be started? There is little definitive information, but on the basis of clinical experience and scattered clinical studies, several guidelines can be suggested. In one controlled study[56] of patients on a pediatric service it was found that discontinuation of antibiotics after defervescence with continued neutropenia often led to recurrence of fever, which then responded to the same antibiotic regimen. The researchers concluded that antibiotics should be continued in these patients until the neutrophil count returned to greater than 500/mm^3. They failed to indicate, however, how soon after defervescence antibiotics were stopped; an alternative approach that we have found useful is to continue antibiotics for 3–5 days after defervescence and then stop them one at a time, 24–48 hours apart, reserving the aminoglycoside to be stopped last.

The situation is quite different in a patient who fails to defervesce. If the patient appears clinically stable and the temperature is no higher, or perhaps somewhat lower than initially, it may be assumed that the antibiotics are suppressing the infection, but in the absence of neutrophils are unable to eradicate it. It is hazardous to stop antibiotics in these patients, but mere persistence of fever does not always indicate the necessity for altering the regimen. At other times, bacterial superinfection may be suspected and the regimen altered accordingly. For instance, several institutions have noted superinfection with *Corynebacterium* CDC-JK,[55,66,78] which may be anticipated by surveillance culturing of long-term neutropenic patients; vancomycin must be added when this organism is suspected. In other situations, it may be known that a certain resistant organism such as *Serratia marcescens* occurs frequently on a particular ward, and failure of a patient to defervesce may prompt inclusion of an antibiotic to cover that organism.

Treatment of Fungal Superinfection

Development of fungal superinfection is suggested by certain clinical pictures, and usually, although not necessarily, does not occur earlier than day 7–10 of treatment with a broad-spectrum antibiotic regimen. Defervescence with subsequent fever spikes several days later, or a stable fever curve with subsequent higher fever spikes, strongly suggest fungal superinfection, particularly if associated with new pulmonary lesions (in aspergillosis) or skin or eyeground lesions (in candidiasis). Unfortunately, none of these is reliably present, and often one must make a decision on clinical grounds along. Autopsy studies have revealed frequent *Candida* involvement of the gastrointestinal tract of neutropenic patients who had been receiving broad-spectrum antibiotics, and as a consequence, we are inclined to start amphotericin B earlier rather than later. Once started, it should be given so that therapeutic doses may be attained within 24 hours: a test dose of 1 mg in 250 ml 5% dextrose in water i.v. over 1–2 hours is given, which, if tolerated, should be followed immediately by increments of 5–10 mg every 6 hours up to 1 mg/kg/day. After the patient stabilizes the drug may

be given every other day at a dose of 1.5 mg/kg. If the patient develops an anaphylactoid reaction to the 1-mg test dose, then 0.1 mg should be given as the first dose, with increments of 0.5–5 mg every 6 hours to attain 1 mg/kg in 48 hours. We prefer to avoid premedication with hydrocortisone (used to prevent fever and chills) insofar as it renders the fever chart difficult to interpret. We have found that meperidine administered during the infusion, when an episode of rigors occurs, is effective in aborting it.

Preventive Measures

Experimental methods such as protected environment,[16,37,79] oral absorbable and nonabsorbable antibiotics,[14,28,70] and leukocyte transfusion[67,72] have been tried in neutropenic patients and found inconsistently effective. Since they involve expense and danger as well as discomfort and inconvenience, they should remain experimental until further studies demonstrate a conclusive advantage.

Attempts at protective immunization have been undertaken against *Ps. aeruginosa*[77] and varicella zoster.[30] The latter seems promising, but the former failed to provide long-lasting immunity and patients' lives were not prolonged. Hyperimmune globulin protects against varicella zoster if given early (within 72 hours) after exposure.[9] Use of prophylactic isoniazid for tuberculosis and presumptive treatment of *Strongyloides stercoralis* with thiobendazole in appropriate patients are effective preventive methods and have been discussed earlier. Live vaccines (measles, rubella, Sabin polio immunization) should not be administered to immunocompromised patients. The preventive measures that are most effective, are applicable to all patients, and are the simplest to provide: hand washing by all personnel between patients, scrupulous care of catheters, frequent changing of intravenous catheters, and general avoidance of manipulative measures whenever possible.

Summary

Patients with lymphatic neoplasms are susceptible to severe and unusual infections by virtue of both their underlying diseases and the therapeutic measures undertaken in their care. Signs and symptoms are often subtle and require a high index of suspicion, since early diagnosis and treatment is of the essence in these patients. The specific etiologic agent cannot always be identified and initial treatment of a suspected infection can often be selected only on the basis of the patient's known immune defects. Several guidelines are offered for initial therapy and, depending on the response, for continued treatment of infection in patients with lymphatic neoplasms. Prophylactic treatment with INH or thiabendazole in selected cases can prevent the development of severe syndromes in patients on immunosuppressive therapy; but the most important preventive measures remain handwashing by personnel, scrupulous care of catheters, and avoidance of manipulative procedures whenever possible.

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CHAPTER 13

Radiologic Approach to Diseases of the Lymph Nodes

Renato Musumeci and Bruno Damascelli

The radiologic study of diseases of the lymph nodes offers a series of procedures for use in rational strategies designed to determine whether or not disease is present, to assess its nature, and to assess its extent. The choice of approach depends on the cost–benefit ratio and on which investigation or series of investigations is considered most likely to answer the relevant questions. Most traditional radiologic procedures furnish indirect answers in that they permit an assessment of the changes induced in the organs or systems under study by neighboring lymph node alterations. Others provide dimensional information only, demonstrating nodal enlargement, often with little or no clue as to the cause. Only lymphangiography, by directly contrasting the intrinsic lymph node architecture, can supply simultaneous information on the presence, nature, and extent of disease. In oncology it is the third point that is most important, because the diagnosis of disease is as a rule suspected clinically, suggested radiologically, and confirmed by histologic and/or laboratory investigations.

In this process the radiologist is responsible for continually comparing the radiologic findings with the clinician's, in order to (a) provide objective evidence for them, (b) suggest surgery, where necessary; (c) suggest the most suitable site for biopsy; or (d) point out the existence of intercurrent disease, a complication or a particularly high-risk site.

Plain Film

Superficial Lymph Node Sites

The diagnostic value of plain film depends upon the body site under study. All the superficial lymph node groups can be examined radiologically, but the findings are usually quite nonspecific and certainly not diagnostic. What we see is a mass of uniform opacity with blurred outlines distorting the normal anatomy of the region and canceling the adipose–fascial and subcutaneous planes. There are no criteria for distinguishing whether a swelling is of nodal or other nature, unless the opacities are due to calcification or ossification within a single node. The pathologic conditions that may lead to calcification or ossification of a lymph node are listed in Table 13–1. All, except tuberculosis, are uncommon.[2,64]

Some workers[43,44,71] suggest using xeroradiography, especially for the axillary region (Figure 13–1). In the experience of Kalisher[43] the signs are as follows:

1. Inflammatory lymph nodes: diameter exceeding 2 cm, densely opaque, with angular and irregular borders
2. Metastatic lymph nodes: diameter exceeding 2.5 cm, dense, with lobulated, infiltrated borders, tending to become matted, with loss of the pericapsular fat line
3. Hyperplastic lymph nodes or nodes with lymphoma: much increased diameter, smooth, with intact capsule and pericapsular fat line.

In one series of breast cancer patients the Kalisher et al.[44] found that the accuracy of xerography in the axillary lymph nodes was 68%, with 29% false negatives—rates statistically similar to those of clinical examination.

Chest

The intrathoracic lymph nodes are among the most commonly affected of the entire lymphatic system both by primary and by secondary disease

TABLE 13–1 Pathologic Conditions with Calcification or Ossification of the Lymph Nodes

Tubercolosis
Histoplasmosis
Ovarian carcinoma metastatic (psammoma bodies)
Osteogenic sarcoma metastatic (ossification)
Irradiated Hodgkin's disease

(Figure 13–2). The technique of chest radiography must be simple but of a high standard in order to extract all the information needed for a correct diagnosis. The minimal approach requires filming in the orthogonal views. Posteroanterior frontal film is undoubtedly the most "legible" if the high-voltage technique and antidiffusory grid are used. This method evidences the various shadows that make up the mediastinal image, and modest variations from the norm suggest lymph node involvement. Tomography should be used at the radiologist's discretion in the assessment of doubtful or vaguely pathologic images in order to confirm or exclude disease, and establish its precise site and pattern for the purpose of differential diagnosis. It is very important to recognize any changes of other etiology in the lungs, pleurae, bronchi, or mediastinum that may explain the enlargement of several node groups. The chest film should always be examined in its entirety and any unusual

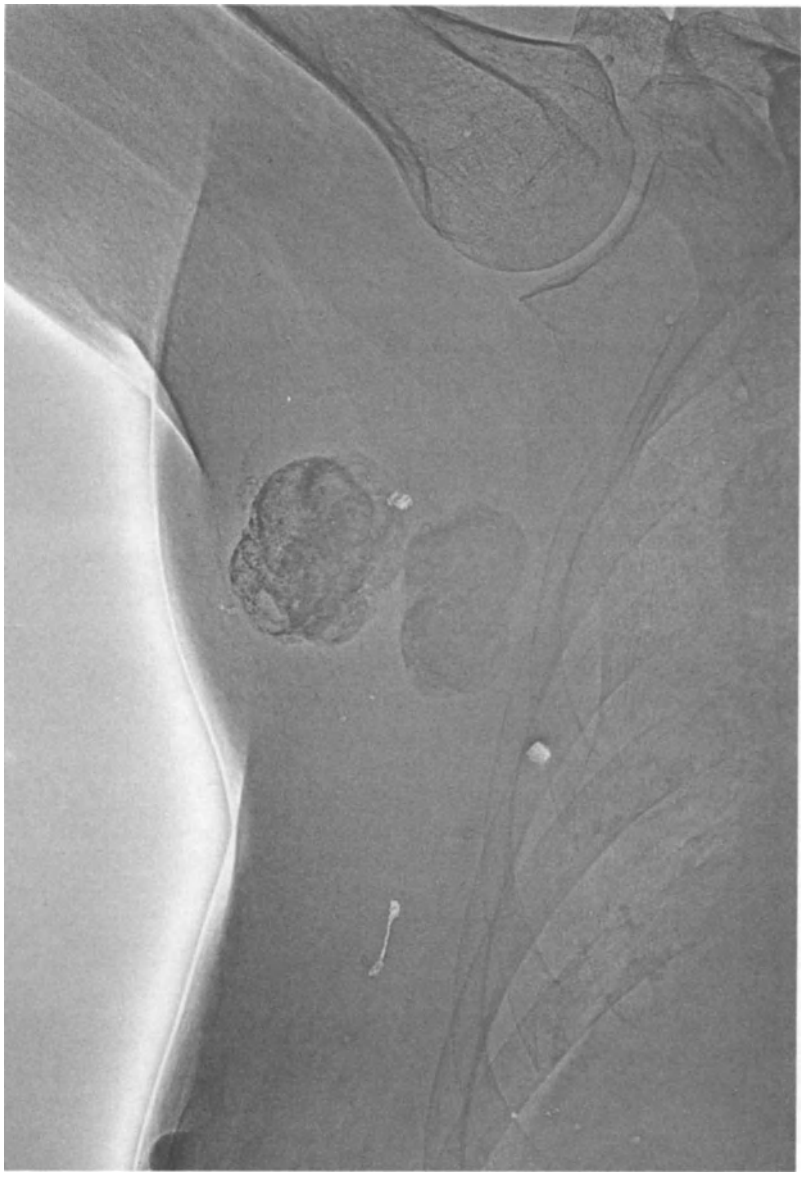


FIGURE 13–1 Xeroradiography of the axilla: calcified metastatic lymph nodes from ovarian carcinoma.

FIGURE 13–2 Neoplastic enlargement of the thoracic lymph nodes in nodular sclerosing Hodgkin's disease. (a) Bilateral involvement of paratracheal and prevascular nodes and left pulmonary root. (b) Enlargement of right paratracheal nodes with bronchial compression and lung hypoventilation. (c) Bilateral involvement of paratracheal, prevascular, pulmonary, and hilar nodes; subcarinal nodes also appear enlarged.

image detected and interpreted. The various pathologic conditions that may lead to enlargement of the mediastinal and/or hilar lymph nodes are listed in Table 13–2.[99] Some workers favor using xerotomography for the simultaneous evaluation of adenopathies and concomitant bronchial or pulmonary lesions,[71] but it has not resulted in signal improvement.

Standard radiography and tomography may be supplemented from time to time by other radiologic procedures, simple or sophisticated, in order to make the diagnosis more specific. These include radioscopy, barium opacification of the esophagus, arteriography or venography, and computerized tomography (CT). These procedures are discussed under separate headings, but it is worth noting here that esophagography is particularly useful in establishing the presence of a subcarinal or retrotracheal mass large enough to alter the course and contour of the esophagus. Paraesophageal, paraaortic and interesophagoaortic swellings can likewise be detected from their repercussions on the contrasted viscus.

The first step in interpreting plain films is to identify the normal and actual positions of the various mediastinal borders. Adenopathies alter the borders, making them either retract or, more often, project. Such findings are not specific for lymph node diseases and may be simulated by mediastinal alterations of other etiology. Furthermore, adenopathies can be detected on plain film or tomograms only when the nodes are large enough to deform the mediastinal borders, which are basically reflections of the pleura on the lung. Thus, according to Blank and Castellino,[7] "in plain film findings we are usually dependent on recognizing distortions of the 'wrapper' (the mediastinal pleura) in order to recognize the presence of a mediastinal mass." CT, on the other hand, demonstrates the mediastinal contents and the presence of lymph node disease, regardless of the state of the wrapper. In disease of the intrathoracic lymph nodes these simple traditional radiologic procedures are still paramount,[7,25,68] for they permit reliable low-cost diagnoses that are easily repeated and compared. Serial follow-up is also easy, provided that every film is of high quality.

Of all the intrathoracic lymph node diseases, the lymphomas undoubtedly have the highest incidence.[7,31,68] In Table 13–3 the results of chest examinations at onset of disease in 200 patients with Hodgkin's disease and non-Hodgkin's lymphoma are presented, showing the high incidence

TABLE 13–2 Mediastinal and/or Hilar Lymph Node Englargement

Common	Rare
Broncogenic carcinoma (mainly undifferentiated)	Fungus disease
	Bacterial infection
Leukemia–lymphoma	Connective tissue disorders
	Histiocytosis X
Metastatic disease	Benign lymphoid disorders
	Parasitic disease
Tuberculosis	Viruses
	Drugs
Sarcoidosis	Agammaglobulinemia
	Erythema nodosum
Pneumoconiosis (egg shell)	Wegener's granulomatosis
	Idiopathic

Adapted from Wood.[99]

of intrathoracic involvement in these diseases, especially Hodgkin's disease, and the various lymph node groups involved. Nodal involvement usually signifies considerable enlargement, which makes detection and diagnosis easy, but some strategic sites or very small nodes can only be picked out by tomography or CT. The possibility of concomitant disease superimposed on lymph node disease must never be ignored: the various intrathoracic signs must be carefully analyzed to exclude or confirm this. In Hodgkin's disease the extent of mediastinal lymph node involvement also has prognostic value,[54] as does hilar involvement.[8] A formula has been devised for measuring this "bulky disease,"[54] which significantly affects survival[54,74] (Figure 13–3).

Abdomen and Retroperitoneum

Plain films of the abdomen may be of some diagnostic value but they are less convincing than chest films; although of some interest, they are not indispensable.[7,18,31,68] Excluding cases of calcification, which have already been alluded to (Table 13–1), there are some very characteristic images suggestive of lymph node diseases. The nodal mass may be identified when it is large enough to deform or obliterate the adipose planes surrounding the various upper retroperitoneal and pelvic parenchymal organs and muscles. Deformation or obliteration of the margin of the psoas and/or displacement of the kidneys, usually upward and outward, may be observed. Gaseous images in the stomach or intestine are likewise displaced. The shape and size of the liver and spleen and the status of the skeletal framework may also be observed on plain film, which thus provides additional relevant information for more precise diagnosis.

Lymphangiography

Lymphoma

Lymphangiography is still an important tool in hematology. Notwithstanding the introduction of ultrasound and CT, it is still essential in the diag-

TABLE 13–3 Chest Involvement in Hodgkin's Disease and Non-Hodgkin's Lymphoma at Disease Presentation

Involvement	Hodgkin's Disease (%)	Non-Hodgkin's Lymphoma (%)
Normal	39	55
Abnormal	61	45
Mediastinal adenopathy	93	82
Hilar adenopathy	33	18
Lung	16	8
Pleura	8	18
Bone	3	2

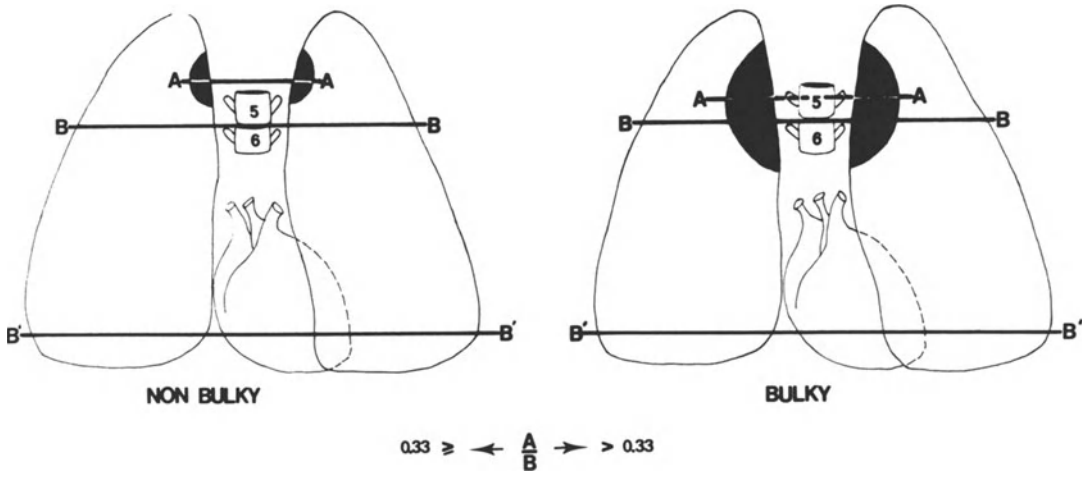


FIGURE 13–3 Mediastinal Hodgkin's disease: nonbulky compared to bulky. **(a)** Scheme for measurement. **(b)** Nonbulky disease. **(c)** Bulky disease.

nosis of Hodgkin's disease. In non-Hodgkin's lymphomas, on the other hand, given the number and incidence of unopacifiable intraperitoneal sites and the size usually attained by these nodes, lymphangiography will probably be superseded by ultrasound or CT.[46]

The lymphangiographic signs on which the diagnosis of lymph node involvement is based are now standardized.[69] The salient findings are as follows:

1. Node size: Although structural changes in normally sized lymph nodes are frequently found, enlargement, often to a marked degree, occurs in 70% of cases of Hodgkin's disease and in 87% of non-Hodgkin's lymphomas.
2. Contrast distribution within the nodes: Altered distribution presents radiologically as foaminess. Although not peculiar to lymphoma, it is fairly typical, especially when associated with enlargement (Figure 13–4).
3. Filling defects: These occur when areas of nodal parenchyma are replaced by lymphomatous tissue, with obliteration or compression of the medullary sinusoids. They are more frequent and pronounced in Hodgkin's disease, especially in the nodular sclerosis subtype.
4. Reticular pattern: In non-Hodgkin's lymphoma the lymph nodes are enlarged and the contrast medium presents a rough and irregular reticular pattern.
5. Status of the lymphatic pathways: In over 60% of non-Hodgkin's patients the lymphatics are perfectly normal. Only 4% present signs of lymph block with collateral circulation.
6. Extent of nodal involvement: Whatever the histotype, non-Hodgkin's lymph node involvement is usually extensive, including several

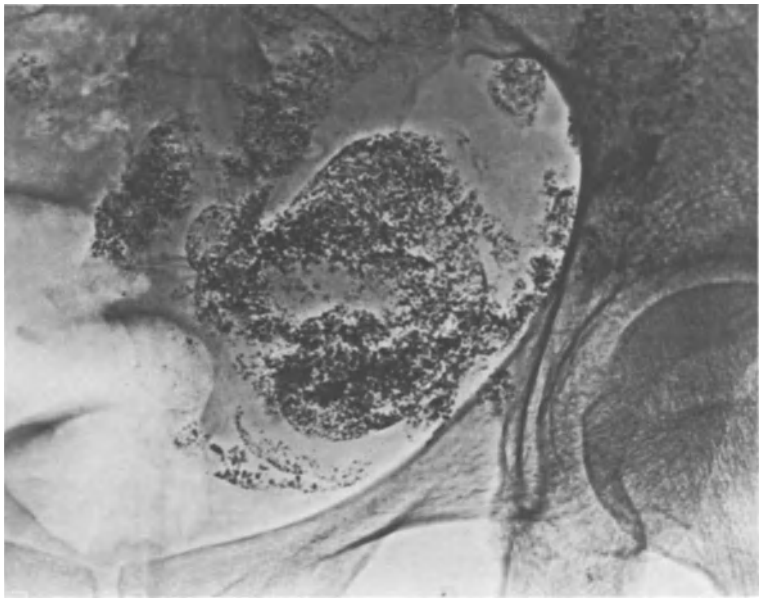


FIGURE 13–4 Direct radiographic magnification of non-Hodgkin's lymphoma demonstrates the typical foamy appearance.

node groups. Topographically, of the various inguinal and retroperitoneal lymph node groups, the ones most affected in Hodgkin's disease are the central nodes in the para-aortic chains. Inguinal involvement is somewhat rarer, except in the predominantly lymphocytic histotype, in which 30% of pathologic cases have such involvement. In non-Hodgkin's lymphoma the incidence of deep central and peripheral involvement is about equal.[68-70,97]

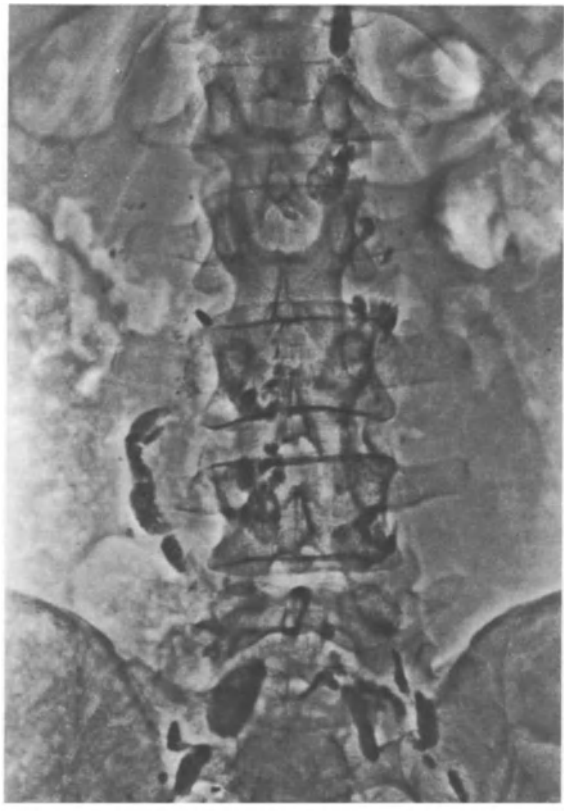
In patients with Hodgkin's disease about 25% of examinations are pathologic at the onset, 16% in supradiaphragmatic clinical stage I and 17% in stage II. The positive rates in non-Hodgkin's lymphoma are quite different: 44%, 29% in supradiaphragmatic stage I and 16% in stage II.[16,69,70]

In patients who have undergone diagnostic laparotomy with retroperitoneal lymph node biopsy the accuracy of lymphangiography is reported to be very high: from 90% to 92%.[18,69] In a recent review of 558 patients, most of whom had Hodgkin's disease, we found that diagnostic accuracy was 96%.[70] Despite the argument between advocates of lymphangiography and those of the newer technologies, it is safe to say that for the lymph node groups that can be opacified lymphangiography still has the edge over CT and ultrasound in detecting the spread of lymphoma;[19] the accuracy rates are 96% for lymphangiography, 85% for CT, and 88% for ultrasound.[19] The two noninvasive methods are obviously in principle superior to lymphangiography in that they permit exploration of regions inaccessible to the older procedure. The present impression is that all three methods are in fact complementary, each making up for the shortcomings of the others and in combination ensuring comprehensive and reliable intra- and retroperitoneal staging of lymphoma (Figure 13–5).

In lymphoma, lymphangiography is still, in our view, mandatory regardless of histology, age, general health, and presentation of the disease. The contraindications are very few, the only important one being cardiac interatrial communication with right-to-left shunt, because of the risk of systemic oily embolization.

Carcinoma

The cardinal radiographic signs of lymph node metastases from a solid tumor may be divided into two groups:[69,93] (a) direct imaging of the metastasis; and (b) indirect evidence from the lymph vessels both close to and distant from the affected nodes. The principal finding is an opacification defect, usually combined with enlargement of the node. The defect is usually marginal or at least eccentric, often with a gap in the contour of the node due to the obliteration of the marginal sinus. The defect is due to the replacement of the lymph node parenchyma by neoplastic tissue impenetrable to the contrast medium. A characteristic sign



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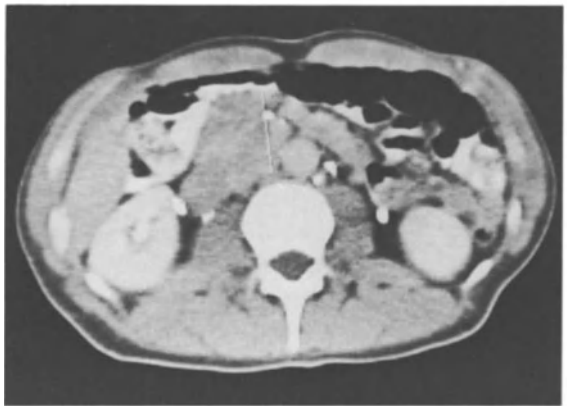

b

FIGURE 13–5 Complementary methods used in the diagnosis of Hodgkin's disease. **(a)** Lymphagiogram showing equivocal involvement of the right upper para-aortic region. **(b)** CT of the same patient showing a huge nodal mass that is not opacified in the same region.

in the early phase is the absence of the radiopaque striations that represent the filling of the lymph node sinus with iodized oil.[69,93]

In addition to direct imaging of the metastasis, indirect signs pointing to changes in the lymph vessels are contributory. The radiologic signs of an obstruction to lymph drainage are

1. Dilatation of the vessels before the obstruction
2. Prolonged and persistent filling of the vessels
3. Perilymphatic extravasation of contrast medium
4. Filling of vessels not normally visible.

Both classes of signs are of equal importance in the diagnosis of metastatic spread and the pattern must be assessed as a whole; the data from both early and late phases should be considered. Knowledge of the nature and site of the primary tumor is essential because information on the lymph drainage of the organ and on the frequency and preferential sites of metastasization may suggest which nodal groups are most at risk and hence require closest attention.

Numerous factors reduce the reliability of the investigation:

1. Lymphangiography does not visualize all the nodal groups that may be the site of metastases.
2. Even in the groups known to be reached by the contrast medium, some may not opacify.
3. To be detected in opacified lymph nodes, a metastasis must have a diameter of at least 3–5 mm.
4. Metastases clearly detectable on the film in contact with isolated lymph nodes may be missed *in vivo,* a difficulty that in some cases can be overcome by using oblique views, direct magnification, or tomography.
5. Alterations of lymph vessels and nodes due to several causes may simulate or mask metastases.

The reported diagnostic accuracy of lymphangiography in solid tumors varies widely from author to author and according to primary site. Clearly, a correct diagnosis requires more than close scrutiny of several films; it calls for a great deal of experience and a thorough knowledge of the natural history and spread of the tumor under study. The diagnostic results obtainable in solid tumors are summarized in Table 13–4. The rate of positive examinations at onset is approximately 25% and the diagnostic accuracy between 85% and 90%. The error margin is made up mostly of false negatives, in the main because of micrometastases (e.g., from cervical carcinoma) or nonopacification of a lymph node or of an entire group (e.g., from testicular cancer).

TABLE 13–4 Diagnostic Results of Lymphography in Solid Tumors

Tumors	No. of Cases	Pathologic Lymphography (%)	Lymphographic–Histologic Correlation (%)
Urologic	981	41.8	85.8
Gynecologic	1630	28.9	86.4
Large bowel	484	19.8	93.7
Skin melanoma	328	40.5	89.7
Bone sarcomas	66	15.1	100 (4/4)
Soft tissue sarcomas	109	25.6	96.3(26/27)

In spite of its undoubted limitations, lymphangiography still retains its major role in the assessment of the N parameter of solid tumors. Once again, no single diagnostic procedure is 100% efficient, but the best screening technique is a combination of lymphangiography and CT. The latter can scan body regions quite inaccessible to the former and visualize large unopacified lymph node metastases, thereby lowering, sometimes dramatically, the false-negative rate of lymphangiography. Another advantage of the combination is that the latter can eliminate some of the overreadings of CT related to nodal enlargement secondary to nonneoplastic disease and also some false negatives in the case of normal-size nodes.

Benign Conditions that May Simulate Neoplasia

A large number of benign conditions may produce a lymphoangiogram similar to those seen with neoplasia. The features that distinguish them are listed below.

1. Fibrolipomatosis: Degenerative-involutional changes of senile lymph nodes are infiltrated by adipose and fibrous tissue. Inguinal groups in particular may present filling defects at their upper poles, crossed by sinusoids in the early phase, not occasioning stasis.[12,20]
2. Lipoplastic lymphadenopathy: An accumulation of normal adipose cells starting from the nodal hilus may be observed, especially in obese elderly women. The lymph nodes will be enlarged with extensive and confluent multiple filling defects but with no signs of stasis and with the marginal sinus free.[98]
3. Hyperplasia: This is one of the commonest lymphangiographic findings and one of the most frequent sources of interpretative error. The lymph node is enlarged with intact marginal sinus, fewer dots (but in large and regular patches), and no noteworthy foaminess. The extent varies but is usually large.[20,69]
4. Acute nonspecific inflammatory processes: Enlarged lymph nodes with frequently dilated sinusoids, capsular involvement, and matting of the nodes are seen. When there is central necrosis the contrast medium is distributed in large irregular lacunae with perilymphatic extravasation into the soft tissues. The afferent lymphatics are often dilated, with signs of stasis.[83]
5. Tuberculosis: The lymphangiographic patterns of this disease cover the whole gamut from signs of more or less overt hyperplasia to multiple filling defects simulating lymph node metastases from epithelial neoplasias. In the course of recovery one may encounter diffuse calcification of all the lymph nodes, which are thus partly or wholly excluded from the lymphatic circulation.[2,75,88]
6. Syphilis and other bacterial infections: In secondary syphilis, leprosy, Nicolas-Favre disease, tularemia, and brucellosis, lymphangiography contributes little. However, when the procedure is done the pattern is of a nonspecific nodal inflammatory process, which in full-blown cases simulates lymphoma.[3]
7. Infestations: Lymphangiography is rarely performed in this group of diseases. Potentially, however, all infestations can result in nodal involvement, and when the examination is done the pattern is tumorlike. Cases in point are toxoplasmosis, African trypanosomiasis, leishmaniasis, *Pneumocystis carinii* infestations, and Bancroft's filariasis.[12]
8. Fungal infections: In these diseases lymphan-

giography is of no practical importance, although there have been some radiologic studies of histoplasmosis.[33]

9. Viral infections: Important for their frequency are infectious mononucleosis and cat scratch disease, but there is no indication for lymphangiography. The reported lymphangiograms were performed as a result of hasty clinical misdiagnoses of lymphoma. The lymphangiographic pattern is of more or less marked nodal hyperplasia with no evidence of malignant changes.
10. Sarcoidosis: The lymphangiographic pattern varies but is very often similar to that of malignant lymphoma. The lymph nodes are always enlarged, with irregular contrast distribution and intact marginal sinus. There have been exceptional cases of severe or total disruption of the nodal architecture but never any changes in the lymph circulation. There is no correlation between the lymphangiographic pattern and the duration of the disease or between the extent of mediastinopulmonary involvement and the extent or type of retroperitoneal involvement.[67]
11. Collagen diseases: The chief feature is enlargement of the lymph nodes with intact contours, extended but uniform distribution of the contrast medium, and no filling defects. There have been descriptions of lymphangiographic results in lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, panarteritis nodosa, dermatomyositis, and scleroderma.[75]
12. Angioimmunoblastic adenopathy: In this autoimmune disease defective regulation of the T cells predisposes to an abnormal proliferative and autoimmune reaction of the B cells. For lymphangiographic diagnosis the following parameters are suggested: accelerated velocity of the lymph circulation; pain on injection of the contrast medium; and fairly regular foaminess of all the lymph nodes, which appear somewhat enlarged without any clear evidence of frank malignancy.[57]
13. Immunologic deficiencies: Lymph node changes in patients with primary immune deficits and autoimmune cytopenia have been described. The nodes are distinctly enlarged, the marginal sinuses are dilated, and the distribution of the contrast medium is fairly uniform.[47,72,96]
14. Nodal extramedullary hematopoiesis: In patients with thalassemia major or myelofibrosis there may be enlarged lymph nodes with multiple filling defects but no signs of obstruction to the progression of the contrast medium.[22] It has been shown histologically that the filling defects are due to the deposition of hemosiderin and to foci of extramedullary erythropoiesis.
15. Pseudolymphoma due to anticonvulsants: The opacified retroperitoneal lymph nodes are uniformly enlarged and their intrinsic structure assumes a reticular pattern with diffuse, small filling defects.[80]
16. Whipple's disease: In intestinal lipodystrophy the lymph nodes are diffusely enlarged and their intrinsic architecture is completely disrupted due to large filling defects.[35]

Ancillary Procedures

Gastrointestinal Examinations

Digestive tract contrast studies have gradually been replaced by more sensitive and direct investigations such as lymphangiography, ultrasound, and CT. Digestive tract contrast findings are essentially indirect; compression, displacement, and, occasionally, infiltration of the wall are seen. The chief merit of this examination, however, is that it permits the diagnosis of a gastrointestinal disease to which the concomitant lymph node pathology can reasonably be attributed.[18,25,68] Relatively early diagnosis is possible in the esophagus and duodenal loop because of their proximity to the lymph nodes. However, even in these portions of the digestive tract the contiguous lymph nodes have to attain some size to be visible; and there is no possibility whatever of differential diagnosis. Even involvement of the mesenteric lymph nodes, often on a large scale (as in non-Hodgkin's lymphoma), is usually missed in contrast investigations of the small intestine, even when performed with modern selective techniques.

There was some interest initially in radiography of the stomach in the lateral view for demonstrating retrogastric adenopathies. Surgery has established, however, that it is a hit-or-miss method. Examination of the rectosigmoid region with a barium enema can theoretically reveal extrinsic compression from the locoregional growth of a solid tumor or from spread of lymphoma; but in

FIGURE 13–6 Retrograde pyelogram of a nonfunctioning left kidney reveals a mass in the lower pole with amputation of the calices and pyelovenous backflow. Multiple rounded compressions on the ureter are due to enlarged lymph nodes. Histologic examination suggested nodular sclerosing Hodgkin's disease.

practice it is of very limited diagnostic value since even quite large pelvic or mesenteric lymph nodes may be completely silent because their prevailing direction of growth obviates repercussions on the large intestine.

Contrast Evaluation of the Urinary Tract

Urography has proved to be relatively insensitive to the presence of retroperitoneal adenopathies until the bunches of lymph nodes become so large that they have repercussions on the various components of the urinary tract.[18,25,68] The kidneys are displaced upward and outward and the lumbar portion of the ureter is displaced outward. In their pelvic portion the ureters are pushed toward the midline, especially in patients with systemic adenopathies. In the case of lymph node metastases from a solid tumor there is less displacement, the main feature being concentric stenosis and consequent hydroureter-nephrosis. Bladder changes caused by unilateral or bilateral involvement may be observed only if the adenopathies are very large (Figure 13–6).

The less frequent findings are obstructive nephropathy secondary to ureteral involvement, renal ischemia with hypertension secondary to arterial stenosis from adenopathy, and nephrotic syndrome secondary to occlusion of the vena cava and/or renal veins, again due to compression of the lymph nodes.

In some cases radiologic investigation of the urinary tract may reveal intrinsic pathology, permitting precise classification of the nodal syndrome. The final diagnosis will depend on examinations utilizing conventional radiologic procedures for the urinary tract (excretory urography, ascending pyelography) and the most up-to-date techniques such as ultrasound, CT, and fine-needle biopsy.

Vascular Procedures

With the establishment of less invasive diagnostic procedures such as ultrasound and CT, the role of vascular contrast studies has been appreciably reduced and individualized. It is rarely possible to demonstrate a disease of the lymph nodes by a vascular procedure, and, when it is, the evidence is mainly indirect. In the arterial system the radiographic signs are a displacement of vessels by direct mural lesions and a more or less pronounced inflammatory or neoplastic circulation. When the veins present signs of compression or infiltration the disease is usually well advanced.

With these reservations in mind, it is worth describing the indications for vascular procedures in lymph node diseases because these procedures still form part of the radiologic armamentarium for other purposes; therefore, skill in performing and interpreting them is still essential. Vascular procedures are in fact being updated to provide information on surgical anatomy and to be used as therapeutic aids (for embolization, regional chemotherapy, and transvascular biopsy).

Peripheral Lymph Node Groups

Arterial catheterization has rarely been requested for determining the status of the cervical lymph nodes. On rare occasions it could be used to investigate suspected intense vascularization or to check

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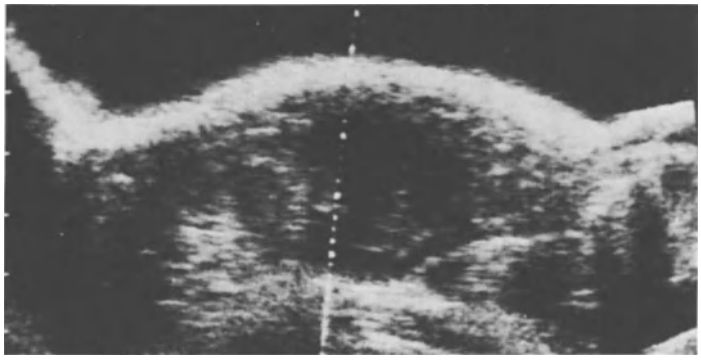

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FIGURE 13–7 Investigation of mediastinal syndrome due to retrosternal metastases from breast cancer. (a) Superior caval venography. The contrast medium was injected bilaterally into the sublingual veins because of the lack of accessible veins in the arm. The right innominate vein is completely obstructed, the left partially. The obstruction was cleared through the cervical veins (direct magnification). (b) Ultrasound scan of the superior mediastinum. The immersion technique was used with craniocaudal inclination. Under the manubrium is a space-occupying lesion that reaches the jugular fossa. The scan permitted measurement of the depth, on the basis of which percutaneous aspiration biopsy was performed (see Figure 13–8).

the possibility of block resection of the carotid axis.[24] Still more rare is the request for venography at sites that are frequently subjected to lymph node resection, such as the lateral cervical, axillary, or inguinal groups. Rarely, one might propose to opacify the internal jugular veins through the sublingual veins when the block resection of these vessels is contemplated.[23]

Mediastinum

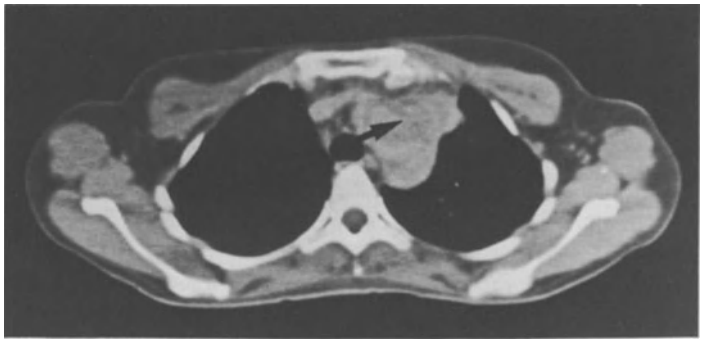
Superior caval venography is indicated for assessing the damage caused by the mediastinal syndrome to which lymph node metastases frequently give rise (Figure 13–7).[27,29] Catheterization of the internal mammary artery may be used as a rare supportive investigation in suspected adenopathies of the anterior mediastinum when a final radiologic diagnosis has not been reached by other means (Figure 13–8).

Abdominal and Retroperitoneal Lymph Nodes

Arterial catheterization to demonstrate a disease of the abdominal lymph nodes must be regarded as quite exceptional and should be considered only when no other methods are available. The accuracy of this examination is very low.[91] This application has rarely been reported, and in most cases it was the result of strategic error or initial misdiagnosis (Figure 13–9). Distinctly more frequent, at least formerly, has been the contrasting of the retroperitoneal venous system—that is, the vena cava, left renal vein, and left spermatic vein—in search of retroperitoneal lymphadenopathies, chiefly in carcinoma of the testis or kidney.[49,58,59,60,63] The technique is in all the textbooks but it is perhaps worth stressing the value of asking the patient to perform a Valsalva maneuver during the search for the junction of the left spermatic vein with the renal vein.

Adenopathies over 2 cm in diameter, as a rule neoplastic, that are to the right of the midline, in front of or behind the vena cava, or between it and the aorta, give rise to the classic pattern of compression of the vessel, or alteration of the profile of its walls in the case of extranodal invasion. Compression must be distinguished from neoplastic thrombosis of the vena cava, which is frequent in tumors of the kidney and is an occasional finding in tumors of the testis (Figure 13–10).

As CT and ultrasound have become more firmly established and more widely available the number



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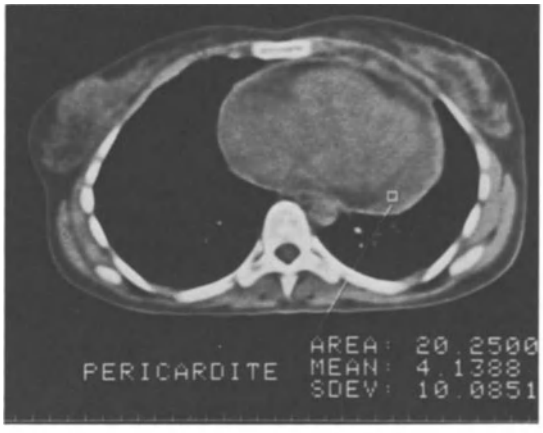

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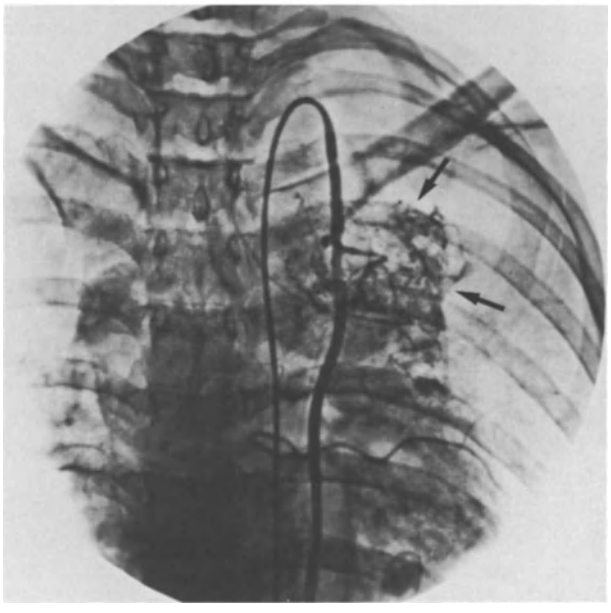

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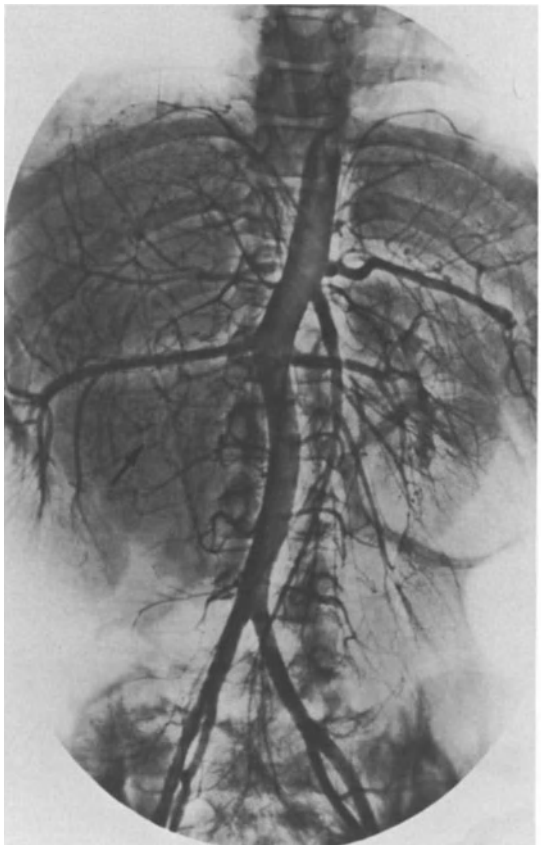
FIGURE 13–8 Investigation of mediastinum in Hodgkin's lymphoma. The patient, with no evidence of other localizations, was admitted for pericardial effusion. The chest x-ray had shown enlargement of the superior mediastinum on the left. **(a)** CT scan of the superior mediastinum confirmed the presence of the lesion, covered by the anterior ends of the ribs and inaccessible to percutaneous biopsy. **(b)** Chest CT scan at cardiac level shows the pericardial effusion. **(c)** Selective angiography of the internal mammary artery confirmed the malignancy of the anterior mediastinal lesion on the left and shows other neoplastic lesions on the pericardium.



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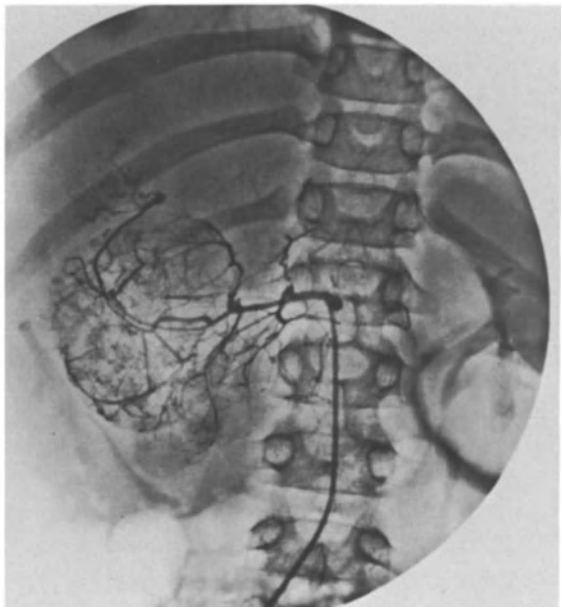

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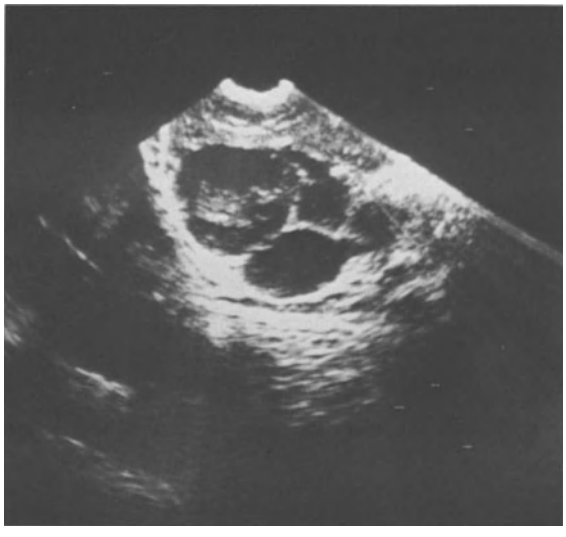

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FIGURE 13–9 Investigation of the abdominal and retroperitoneal lymph nodes in a 10-year-old girl with Hodgkin's lymphoma. **(a)** Abdominal aortography for suspected neuroblastoma. The right kidney, abdominal aorta, mesenteric artery, and hepatic artery are displaced and a neoplastic circulation is visible in the right paravertebral region. There were no calcifications in the preliminary films. **(b)** Selective angiography of the first lumbar artery on the right. The angiographic pattern could be that of a retroperitoneal lymphadenopathy. The subsequent biopsy showed that the condition was multiple lymphadenopathies from Hodgkin's disease. **(c)** Longitudinal abdominal ultrasound scan to the right of the midline. The scan yielded the diagnosis of right retroperitoneal Hodgkin's lymphoma more simply and with greater precision than the arterial catheterization procedures.

of inferior caval, renal, and spermatic venographies for the diagnosis of retroperitoneal adenopathies has declined. This evaluation applies to surgical patients with large adenopathies secondary to carcinoma of the testis or kidney. However, left renal venography and spermatic venography must be considered complementary to and/or competitive with lymphangiography in carcinoma of the testis.

It is not always possible to demonstrate involve-

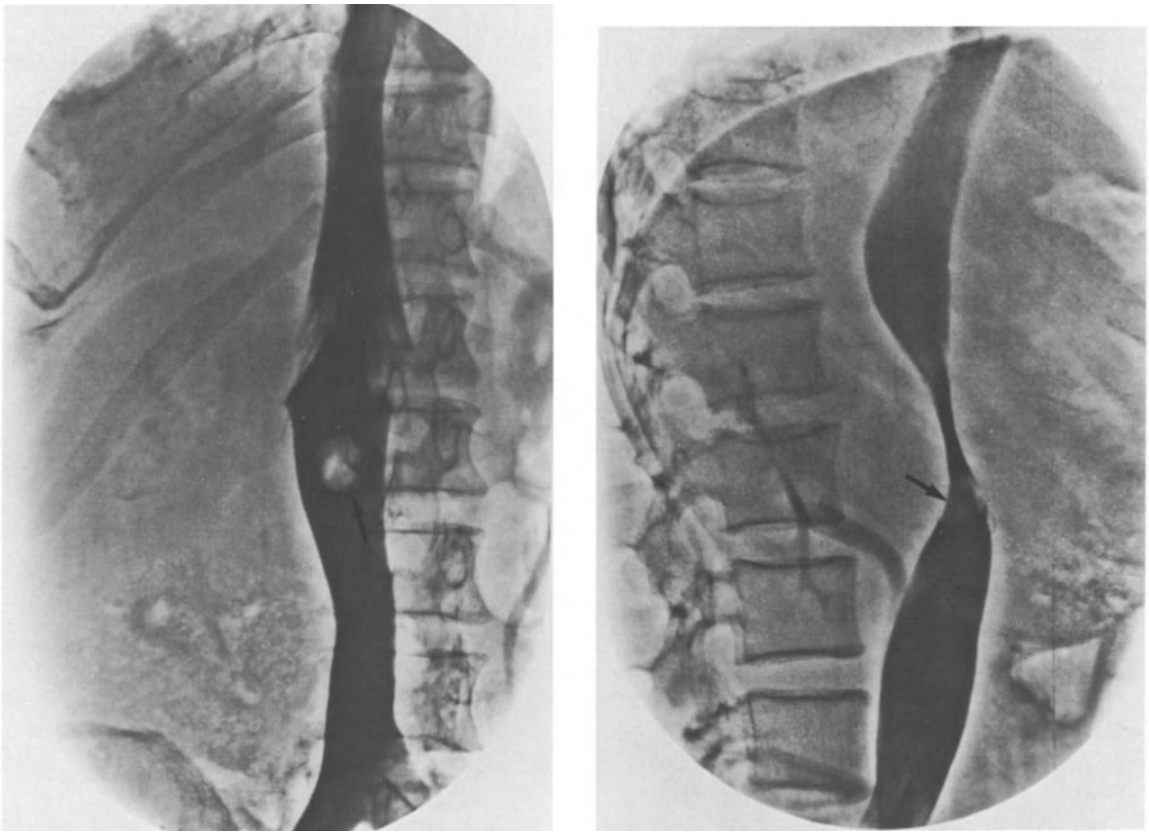

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FIGURE 13–10 Neoplastic thrombosis in the vena cava from carcinoma of the testis. **(a)** The thrombus, detached from a lymph node metastasis, has reached the vena cava via a lumbar vein and appears as a filling defect in the lumen of the vessel. **(b)** Inferior caval angiography in the lateral view. The neoplastic thrombus is still visible, as are the anterior displacement of the vena cava and the reduction of caliber which was not apparent in the frontal view. The displacement was caused by the lymph node metastasis.

ment of the left superior para-aortic central node or group because either at the time of staging the patient has already undergone orchiectomy, or lymphangiography has not reached the superior para-aortic groups. Furthermore, considering the nonspecificity of CT and ultrasound in the assessment of remission of lymph node metastases from carcinoma of the testis after chemotherapy or radiotherapy, and the possibility of mistaking them for intestinal loops, there are good reasons for retaining venography in the diagnostic repertoire.

Ultrasound

The saying that there is nothing absolute in medicine applies with particular force to the clinical use of ultrasound. During its development, especially swift in the past 5 years, ultrasound has encountered both scepticism and enthusiasm among medical professionals, in some respects surpassing and in others falling short of expectations. As a means of displaying anatomy, normal or pathologic, ultrasonography is easy to use, safe, cheap, and applicable to all age groups, but it's usefulness still depends unduly on the operator's skill. If the technique is an integral part of a wide array of facilities, with all the other imaging techniques available at the same medical center, it should be fully accepted. At some sites it is valuable as a primary means for guiding the biopsy of deep-seated tissues; at other sites it is a useful alternative.

Ultrasound applications have developed along three lines: high resolution; evaluation of blood flow both in vascular trunks and tissues; and tissue

characterization. At present only the first feature has been exploited clinically to any marked degree; and we think that, at appropriate sites, it is useful in investigating diseases of the lymphatic system.[21,56,78,82]

Peripheral Lymph Nodes

When ultrasound is used to study superficial lymph node groups (e.g., the lateral cervical, supraclavicular, axillary, and inguinal node groups), the real-time technique with small transducers is the most suitable. Until the application of ultrasound for tissue characterization is developed further, the information gained by its use is of limited clinical value because all these groups are accessible to physical examination and surgical biopsy. At present the technique is of most interest because it can be used to guide percutaneous biopsy. Fine-needle aspiration may be recommended even for clinically accessible nodes when it is necessary to check the course of a known disease or when numerous confluent nodes or inflammatory adenopathies are present. In these circumstances surgical biopsy may be postponed until after reductive therapy, based on the histologic and bacteriologic evidence obtainable by percutaneous aspiration.

Mediastinum

Ultrasound demonstration of lymph nodes in the mediastinum is obstructed by the sternum and rib cage, and by the air in the lungs. By individualizing the approach in various situations and using the jugular fossa and/or intercostal spaces as portals of entry, one can use ultrasound to advantage. In these cases ultrasonography can be used to get an idea of lesion thickness and of the depth at which the biopsy may be performed. Differentiation from a vascular structure is facilitated by the use of a two-dimensional doppler, but ultrasound alone can sometimes establish the characteristics of a space-occupying lesion.

The advantage of ultrasound over x-ray examinations for guiding a biopsy is unquestionable. This technique should be attempted at least in lesions of the anterior mediastinum that project from the sternal margin and that therefore fall within the range of vision of a small modern transducer, for which there is room in the intercostal spaces or jugular fossa. Although CT is more anatomic, is more complete, and can guide a biopsy without exposing the operator to the x-ray beam, the following points must not be forgotten: the patient is exposed to further radiation; the respiratory movements necessitate more than one thin-layer scan for the exact positioning of the tip of the needle; the needle is unlikely to enter any lesion perpendicularly; frequent rectification is necessary, with the needle passing through the same point several times; the needle remains in the tissues for a long time; and the sum of these disadvantages increases the risk of complications.

Abdominal and Retroperitoneal Lymph Nodes

The demonstration of abdominal adenopathies is practically confined to the para-aortic and iliac chains, because at other sites it is more difficult to find anatomic landmarks and a single enlarged lymph node is indistinguishable from any other solid space-occupying lesion. The technique of demonstrating the retroperitoneal lymph nodes is not simple. The main advantages of ultrasound are that sections can be made in all planes and the distance between sections can be graduated infinitesimally. The ease with which ultrasound can be used to keep track of an anatomic structure and image it in practically all its planes is often offset by insurmountable obstacles, such as the presence of gas in the alimentary canal, the thickness of the abdominal wall, and a robust body build.

Before a retroperitonal examination, the patient should be kept on a low-fiber diet for at least 3 days and be given an enema. The patient must present fasting and with a full bladder. The patient is laid on the examination table with support under the back to accentuate lumbar lordosis and thereby bring the spine closer to the anterior wall of the abdomen. Having chosen a transducer of the right frequency and focusing for the patient's thickness, the ultrasound operator locates the aorta and vena cava in longitudinal sections, and then again in transverse sections along with the hilus renalis, the superior mesenteric artery, and the pancreas. Heavy mineral oil should be used for acoustic coupling in preference to water-soluble gels, especially for the hairy parts of the body such as the lower pelvis. The pelvic lymph node chains should be studied with longitudinal, oblique, and transverse cuts using, where possible, the acoustic window

of the bladder, overdistended either by voluntary control or by catheterization.

Normal lymph nodes are not usually imaged by ultrasound and hence structures that look like lymph nodes (either singly or in bunches) appearing on the screen are very likely to be pathologic. The smallest nodes that can be imaged with 3–3.5 MHz transducers in patients of average thickness measure about 2 cm. The internal architecture of the nodes may be classified as echo free, weakly or intensely echogenic, and with or without posterior enhancement. Another criterion is the response of the borders of vascular structures (e.g., the aorta and vena cava) adjacent to the enlarged lymph nodes. The scan of an echo-free node without posterior enhancement, or with it at a given frequency, must be compared with at least one other at a higher frequency with precise focusing for that depth. The intrinsic retroperitoneal lymph node signs are few and, other than the features mentioned, there are no criteria for correlating an adenopathy with a given histologic type. It is said that Hodgkin's disease presents relatively transonic adenopathies associated less frequently with posterior enhancement and that they can partially blot out the walls of the aorta or vena cava through sound absorption, but assertion is not necessarily true, as may be seen from the numerous examples shown of retroperitoneal metastases from several types of neoplasia, including Hodgkin's disease. The possibility of identifying a non-Hodgkin's lymphoma is very good when the nodes are large and intrinsically echogenic, but even this finding has to be correlated with the clinical history and all other preliminary data.[10,48,77]

Since most workers agree that there is little point in expecting ultrasound to deliver an image of lymph nodes outside the common iliac and paraaortic groups, the following suggestion is made: if the patient has had a lymphography it is useful to do a quick fluoroscopy in order to have a skin marker of the level reached by the iodized oil and thereby concentrate attention on the groups not covered by lymphography. If the latter has located node lesions that can be biopsied, and if the affected nodes are large enough, during fluoroscopy a skin marker is added for these groups. A subsequent check on the possibility of obtaining a histologic sample is made using ultrasound rather than fluoroscopic guidance, because the large number of biopsies usually needed in such cases raises the problem of exposure.

At the pelvic level interpretation is difficult because an enlarged lymph node may be confused with an intestinal loop, with the colon, or with adnexal diseases in women. As with the retroperitoneal nodes, the pelvic nodes, whether pathologic or normal, should be checked on another day.

Despite these procedural drawbacks and intrinsic limitations on the applicability of ultrasound to the diagnosis of abdominal lymph node diseases, this technique has substantial value in the guidance of multiple biopsies of the lymphatic chains and as a less invasive means of keeping track of a node disease already demonstrated by lymphangiography or CT. The imaging of spread to other organs or to the nodal spaces is a further advantage over conventional radiography, shared with CT, and is discussed in the next section.[13,52,92]

Computerized Tomography

Since its introduction, CT has achieved such a high standard that no radiologic procedure can surpass its imaging of the intrinsic architecture of organs and systems. At the present stage of technologic development, now that high-speed scanners have become available, there is little reason to hold back on the use of CT. It has proved to be of immense value in the study of space-occupying lesions of many organs and systems. Experience has also demonstrated that CT need not be limited to adults and persons of robust body build.[9,90]

The history of CT in the diagnosis of lymph node diseases has been very similar to that of ultrasound. The disadvantage of not being able to take body sections in all planes, as ultrasound can, is to some extent overcome by multiplane reconstruction. At the level of the trunk reconstructed CT images have sufficient resolution in the coronal and sagittal planes only for large lesions in sectors with clear anatomic landmarks. Employing x-rays, CT does not encounter "physical barrier" difficulties, as happens with ultrasound (which is totally reflected by gas or partly or wholly absorbed by tissues). In addition, its density analysis is incomparably superior to that of conventional radiology and keenly competitive with that of gray-scale ultrasound (whose application for tissue classification is still at the theoretical stage) and nuclear magnetic resonance (whose clinical development is expected to take at least another 2 years).

Peripheral Lymph Node Groups

Theoretically, all the lymph node groups are suitable for study with CT. In practice, the procedure has been used mainly for the chest and abdomen because other sites are more accessible to physical examination; an exception may arise from the need to check the peripheral nodes for a specific disease, i.e., for a particular change in tissue density. A lymph node metastasis whose density values reflect the primary tumor histotype can be diagnosed with certainty without invasive investigations.

Mediastinum

A completely different situation obtains in the chest and abdomen, where investigation for adenopathies has always been problematic and at one time entailed the use of several procedures. In the chest CT is unquestionably superior to conventional radiology, including tomography. With the scanning times now available (5–2 seconds), altered lymphatic structures of 1.5 cm or more can be distinguished from the intricate mediastinal anatomy, especially after generous parenteral injection of contrast medium. Key mediastinal lymph nodes can be imaged by axial cuts. Despite its invasiveness (ionizing radiation and contrast medium), CT is also indicated as the procedure of choice for checking the course of a neoplastic disease and as a means of obtaining biopsy specimens.

At present the only contraindications and constraints on CT scanning of the mediastinal spaces are the patient's inability to maintain the horizontal position, extreme stages of respiratory or cardiac malfunction, intolerance to the contrast medium, and the special problem of the anterior mediastinum in young children. It is the persistence of the thymus in youngsters that complicates the differential diagnosis of adenopathies.[5,11,51,65,90]

CT of the mediastinum supplies a series of 5–8 mm contiguous scans from the neck to the ensiform process in two stages, before and after parenteral administration of contrast medium. Simultaneous use of the veins of both arms, faint contrasting of the esophagus with viscous barium, combined use of infusion and bolus injection, and breath-holding, facilitated by the nasal administration of oxygen, complete the protocol for a successful examination even in difficult patients.

As normal mediastinal lymph nodes are not visible, a CT image of 1.5 cm or more in the presumed site of the lymphatic chains definitely denotes abnormality or disease. There are few density changes: an enlarged lymph node is usually of homogeneous density unless there is a metastasis from a known primary tumor and a previous CT scan has supplied an identical image (Figure 13–11). Changes in the CT pattern of a disease during the posttherapeutic course are even harder to assess in the mediastinum than in the retroperitoneum. Necrosis in the mediastinal nodes is rarer and fibrosis less distinguishable. A verdict of regression or regrowth is based on the size of the lesion and on its capacity to accumulate contrast medium (to be studied, where possible, with the dynamic flow technique).

Abdominal and Retroperitoneal Lymph Nodes

The value of CT in the diagnosis of abdominal and retroperitoneal lymph node diseases has been substantiated by an impressive body of clinical ma-

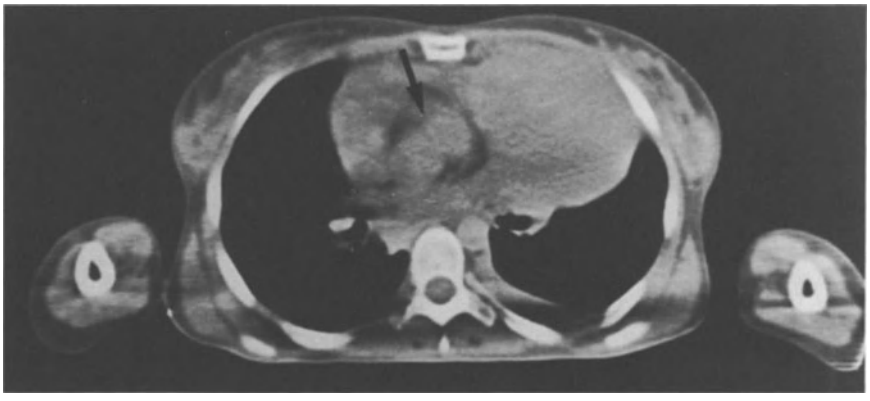

FIGURE 13–11 CT scan of Hodgkin's disease in the mediastinum. Two medial lymphadenopathies are clearly visible just below the hilus.

terial reported by several investigators.[1,4,6,14,15,17,26] There are at least three reasons radiologists have done more with CT in this area than in the mediastinum: (a) the abdominal and retroperitoneal lymph node groups are more numerous and less accessible to physical examination or to rapid radiographic inspection (e.g., a plain x-ray in the case of the chest); (b) the frequency of abdominal lymph node malignancies is higher than at other sites, for in addition to systemic lymphatic diseases there are metastases from genitourinary tumors and tumors of the locomotor apparatus of the lower half of the trunk and lower limbs; (c) the competition from bipedal and funicular lymphangiography, ultrasound, venography, retroperitoneal scintigraphy, and intravenous urography, all in continued, albeit individualized, use.

The relative advantages and disadvantages of the various procedures for the diagnosis of abdominal lymph node diseases are well known and continually written about in the literature, sometimes with drastic value judgments. If the history of radiologic technology in the past few years has taught us anything, it is that it is best to be somewhat cautious in recommending any diagnostic procedure as the method of choice. CT—even with striking advantage of three-dimensional density images over conventional radiography with its compressed images on plain film—does not solve all imaging problems, especially in the case of small structures such as the lymph nodes. It is well to remember that conventional radiology offers spatial resolution of a far higher standard than can at present be obtained with CT and ultrasound. The most recent advances in radiography, including very high resolution fluoroscopy and new versions of direct magnification, afford pictures of the structure of an oil-filled lymph node that are authentic macroradiograms. Filling defects of barely 1 mm are therefore visible, and normal-size lymph nodes are accessible to fluoroscopic biopsy.

The CT examination protocol for the abdominal and retroperitoneal lymph nodes is similar to the one recommended for the abdomen generally. As CT can demonstrate a primary extranodal disease or the spread of a nodal disease to other organs such as the liver or spleen, the situation of what we may call "extended" vision has to be considered from the procedural point of view. Contiguous scans from the ensiform process to the symphysis pubis should be done before and after parenteral enhancement; the patient is prepared with dilute contrast medium at least 40 minutes before. Just before the second round of scans the patient is given a booster of oral contrast medium for the stomach, duodenum, and first loops of the jejunum. We recommend injection of the contrast into the femoral vein, which is accessible percutaneously to commercially available needle cannulas in all age groups. Fast infusion, with fast injection for the critical sections, ensures preferential opacification of the vena cava, which is best exploited as an anatomic landmark, especially for the pancreatic region. In this way direct involvement of the vessel is dramatically obvious, whereas with the customary practice of using the elbow veins the obligate position of the arms in the scanner tunnel can give rise to appreciable variations in the rate of administration of the contrast, with the result that the distribution of density between vessels and parenchyma is too uniform.

In the case of pelvic examinations the patient should not void the bladder beforehand because the distended bladder raises the intestinal loops. Furthermore, since the presence of urine makes for dilution of the contrast medium excreted by the kidneys, artifacts due to density differences are reduced. The same artifacts are often not seen after lymphangiography or by the metal clips used in surgery. Lymphangiography seems to alter the density and volume of the lymph nodes for several days and, when possible, should be postponed until after CT scanning of the abdomen, or even dispensed with if CT is used to probe for retroperitoneal adenopathies in a patient with a histologic diagnosis of malignant lymphoma. In all other conditions the two methods are complementary both for topographic reasons and for their difference in resolving power.

In particular, CT can image the internal iliac chains when pathologic, the para-aortic chains even when normal (above L-1 and below the pillars of the diaphragm), and the mesenteric and celiac chains when pathologic. With scanning times of not more than 5 seconds no other steps are usually necessary. Longer times may require blocking peristalsis with glucagon, which is preferably perfused rather than injected in a single dose. The windows and levels vary according to whether the retroperitoneum, liver, or spleen is to be observed, especially after enhancement.

The CT features of the retroperitoneal lymph nodes are few. As a rule, the spatial resolution,

even with the best scanners, is such that if the nodes are smaller than 1.5–2 cm no reasonable interpretation is possible. If numerous nodes of this size or larger are observed, the situation is definitely abnormal. Adenopathies obliterate the perivascular adipose spaces and the subcrural ones in the high groups, but they are very hard to detect in the pelvis. Large adenopathies displace and deform the blood vessels, and in this connection multiplanar reconstruction on the planes of the great vessels can be useful. If malignant lymphoma is suspected, note that large adenopathies of uniform density are more frequent in lymphosarcoma and non-Hodgkin's lymphoma. Smaller ones of uniform density, chiefly para-aortic, are said to be typical of Hodgkin's disease. Some variation in CT pattern is encountered in metastases from tumors of the testis, depending on the histology of the primary tumor and the therapeutic response. As a rule, adenopathies secondary to systemic lymphadenitis diminish or disappear when responding to treatment, whereas secondaries from tumors of the testis may diminish and be present associated retroperitoneal thickening or appreciable density variations due to necrotic phenomena.[32,40–42,45,50,55,62,66,76,81,84–87,89,94,95,100]

Lymph node metastases from carcinoma of the hilus renalis raise a peculiar problem: they are easily mistaken for neoplastic permeation of a vein. Uptake of the contrast medium is no discriminant if the lymph nodes, riddled with the neoplasia, are hypervascularized, as often happens also in the case of a neoplastic thrombus in the renal vein or vena cava. In children CT presents pecularities in the case of the retroperitoneal as well as other lymph nodes. Nodes measuring less than 1–1.5 cm are not detectable, as in adults. Movement artifacts are a bigger problem and differential diagnosis from lymphoma must take account of the sinuses, immunodepression adenopathies, and retroperitoneal fibrosis.

The role of CT in percutaneous biopsy of the lymphatic system, already mentioned in passing, is now covered in detail. It is worth noting first, however, that the value of CT as a guide to the retroperitoneal lymph nodes is confined to nodes of some size or contiguous nodes or sites not accessible to lymphangiography. Although CT is said to be preferred over other procedures for biopsying smaller lesions, experience has shown that biopsy with CT is a lengthy business and the results are often disappointing. The abdominal lymph nodes are deep seated and the progression of a 22- or 23-gauge needle through the abdominal contents is rarely rectilinear.

Fine-Needle Biopsy

Fine-needle percutaneous biopsy has long been in the armementarium of instrumental diagnosis and in experienced hands it seems to be very servicable for several organs. The method has been said to be relatively noninvasive and virtually free from complications, but as the number of these biopsies increases the complication rate will probably rise too and perhaps the time has come to reassess the indications and the effective risks.

In the chest, often emphysematous, fine-needle biopsy can cause pneumothorax, calling for surgical drainage. Rarely does this accident occur in biopsies of the anterior mediastinum that project beyond the sternal line and make contact with the chest wall. Pneumothorax is a more frequent mishap of pulmonary lymph node biopsies. At the hilum of the lung a potentially dangerous hemoptysis may occur if the blood coagulation status is altered. In a series of 300 lung biopsies checked by the author and carried out with a 22–23 gauge needle, a small pneumothorax was observed in at least 50% of cases. Only 5 patients needed drainage and all presented a prebiopsy situation favorable to pneumothorax, such as emphysema or cough, and most had undergone multiple biopsies deep in the lung parenchyma.

In the abdomen the risk of complications seems to depend on the organ or region being investigated. One death from an acute exacerbation of chronic relapsing pancreatitis was reported recently, and previously there had been a case of needle-track spread from carcinoma of the pancreas.[28,30] We must add another death from transhepatic percutaneous cholangiography performed with a 23-gauge needle in a single run, which we class with aspiration biopsy. The patient, suffering from cholestatic jaundice, had depressed blood coagulation, and his death from hepatic coma was attributed to a large collection of blood and bile under the capsule and within the liver parenchyma. A case of heavy peritoneal lymphorrhea was observed in another of our patients, who had been subjected to fine-needle biopsy at the level of a lymphangioma not recognized as such

beforehand. A third patient in our series, who had undergone a para-aortic lymph node biopsy high in the right peritoneum, was found at subsequent laparotomy to have a clinically silent peritoneal hemorrhagic suffusion. In this case the coagulation status was normal.

In fine-needle biopsy in the pelvis, in cases in which lymphangiography had not been performed and ultrasonography had been used to guide the biopsy of lymph node masses, the tip of the needle often penetrated the bladder, overdistended to serve as an acoustic window. A macroscopic but short-lived hematuria occurred in a child with a recurrence of pelvic rhabdomyosarcoma diagnosed by transvesical biopsy with a 23-gauge needle.

Fine-needle percutaneous biopsy requires few instruments and the maneuvers are simple. Standard Chiba needles of variable length, normally available in 22 and 23 gauge, are used. Apart from the guidance system, the procedure involves the positioning of a brake on the needle to stop its passage through the tissues at the desired depth. With CT the brake should be made of plastic. The syringe should be large (20–40 ml). A pathologist should be on hand to make a supravital diagnosis or to supervise the preparation of the cytologic and imbedded material. Manual aspiration with the syringe is laborious and a special handle should definitely be used. The number of samples taken depends on the gross appearance of the material and on common sense. The needle track should be different for each sampling and aspiration should be stopped on withdrawal of the syringe. Labeling of the sampling site with contrast medium is unnecessary and potentially dangerous.

Peripheral Lymph Nodes

For the lateral cervical, supraclavicular, axillary, and inguinal lymph node groups, which are easily accessible to physical examination and surgical intervention, percutaneous biopsy is rarely indicated and is far more appropriate for a bacteriologic than a histologic examination. There is no point in coping with problems of histologic interpretation (diagnosis of lymphoma) raised by the particular sample gathered by a fine needle when surgical biopsy at these sites presents no difficulty. The help provided by noninvasive centering (e.g., by ultrasounds) has already been discussed.

Mediastinum

As explained earlier, lymph node biopsy in the mediastinum is made difficult by the sternal and costal barrier. The jugular or intercostal portals of entry, under fluoroscopic and/or, occasionally, ultrasound or CT guidance, may permit biopsy of the lymph nodes of the anterior mediastinum. For the deeper nodes fluoroscopy,[34] if necessary biplanal, is preferable. A preliminary estimate of the depth may be made from the chest x-ray and, if monoplane fluoroscopy is used, the operator ascertains whether the needle tip has reached the lesion by placing the patient in the oblique position.

We have mentioned that CT is theoretically more precise, does not expose the operator to radiation, and perhaps exposes the patient less. Scanning at low milliampere values to reduce exposure does not seem worthwhile when a fine needle is used, because it degrades the image unduly. CT biopsy is nevertheless more time consuming, it necessitates keeping the needle within the lung parenchyma or against the structures of the hilum for a potentially dangerous length of time, and the respiratory movements and cardiac activity make it difficult to ascertain whether the needle really is in the lesion. Finally, correction of the trajectory is awkward in the confined space of the scanner tunnel.

Abdominal and Retroperitoneal Lymph Nodes

The abdominal lymph node chains may be more suitable for fine-needle biopsy with a potentially lower risk provided that the liver, pancreas, and spleen are not included in the track. The centering problems are greater than in the chest, the methods of guidance are different, and the lesions as a rule are larger. Fluoroscopy may be used in the upper abdomen for the celiac and mesenteric lymph node groups, contrasting the stomach, duodenum, and first jejunoileal loops as for the digestive tract. This is a possible alternative to the other methods of centering when the lymph nodes are large. If ultrasound and CT are available, they have the advantages of greater safety, greater precision, and more accurate evaluation of the most suitable point for percutaneous aspiration within a given lesion. CT establishes areas of greater or less vascularization by the density of the scans after enhancement.

In the case of a poorly vascularized lesion in direct contact with the abdominal wall, especially if posteriorly, larger gauge needles may be used for biopsy with greater chances of arriving at a final histologic diagnosis.

Fine-needle biopsy of lymph nodes under 2 cm in diameter or even of normal size is possible in practice only if the groups are opacified with iodized oil. Several studies have shown that lymphangiography plus percutaneous biopsy increases diagnostic accuracy in investigations for metastases from genitourinary epithelial tumors.[36–39,53,61,73,79,101,102]

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CHAPTER 14

Chromosome Abnormalities in Malignant Diseases of the Lymphatic System

Lore Zech

In recent years, a considerable amount of information about chromosome anomalies in relation to different malignancies has been accumulated. Prior to the introduction of banding methods, many authors suggested random distribution of chromosome abnormalities in malignant disorders; the Philadelphia chromosome (Ph^1) in chronic myelogenous leukemia was the only exception. Later, however, analysis of banded karyotypes from malignant material gave clear evidence for the occurrence of nonrandomly distributed chromosome changes in most tumors studied thus far, and today it is well established that specific karyotypic anomalies are associated with tumor development.[107]

Lymphomas are second only to leukemia in being the most extensively cytogenetically studied malignancies; one reason for this is probably the availability of material. Lymph nodes, bone marrow, and spleen—the main sources for study of lymphoproliferative diseases—are soft tissues from which cells to be analyzed are easily dispersed. On the other hand, we must admit that, compared with chronic myelogenous leukemia, our knowledge of karyotypic changes in lymphomas is much more limited. We can find several explanations for this: The histopathologic characterization of different types of lymphomas is often incomplete, and the use of different nomenclature by different authors is complicating the situation, making it almost impossible to get accurate information about the exact number of different cases studied. Furthermore, there are technical problems. The chromosome rearrangements can be very complex, making it difficult to distinguish between primary and secondary changes and to identify all markers. In spite of these problems, a clear pattern of specific chromosome changes can be recognized. The main purpose of this chapter is to draw attention to those consistently occurring marker chromosomes because they probably can be regarded as being of essential importance in the origin of lymphomas and malignancies in general.

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I want to express my special thanks to Mrs. C. Björklund and Mrs. B. Stellan for excellent technical assistance.

Procedures and Nomenclature

The results reported in this chapter are mainly based on the study of tissues directly involved by the disease, i.e., lymph nodes, bone marrow, spleen, and peripheral blood without phytohemagglutinin (PHA) stimulation. In some special cases B cells were stimulated by polyclonal B cell mitogens. Effusion material has also been used with rather good results but was not always available.

Chromosome Preparation

For many years it was routine in most laboratories to analyze chromosomes either directly or after 24–48 hours in culture because it was generally accepted that direct preparations were the best source for studies of tumor chromosomes. However, it now seems that assumption is not quite correct. Recently, it was observed that under certain circumstances short-term cultures of bone marrow from patients with leukemia increased the likelihood of detecting malignant cells that had

not been found in direct preparations. It has been suggested that as many as 15% of patients who do not have abnormal karyotypes in the directly prepared metaphases reveal a malignant clone with characteristic tumor chromosomes after 24–48 hours in culture.[8,13] The explanation for this unexpected result seems to be that metaphases seen in direct preparations are erythroblasts, whereas cells dividing after 24 or 48 hours in culture are the leukemic granulocytes.[8] These observations are probably of great importance for cytogenetic evaluation of at least certain types of malignancies and also for routine handling of tumor material.

In some cases it can be difficult or even impossible to analyze primary lymphomas due to the limited amount of neoplastic tissue and the low number of metaphases. Therefore, many studies are performed on lymphoid cell lines. Unfortunately, results from such investigations cannot always be correlated with data obtained from primary tumors from which the lines were established because many changes are of secondary character, caused by culture conditions.

Chromosome banding methods are essential to the cytogenetic study of tumor cells. Many metaphases that appear normal after a conventional nonbanding technique may in fact exhibit unexpected rearrangements after banding. The data referred to in this chapter are based on banded karyotypes only. The recent review by Sandberg includes studies done before the innovation of banding methods.[106]

Banding Techniques

Chromosome banding means the use of special staining techniques by which a series of bands are produced along the metaphase chromosomes. Banding methods, developed since 1968[14] and first applied to the human karyotype in 1969,[129] have lead to the identification of all human chromosomes and have also clarified many structural rearrangements that could not be analyzed with conventional methods.[15,129] Each individual chromosome has its own characteristic banding pattern. According to definition, a band is part of a chromosome clearly distinguishable from adjacent parts by appearing lighter or darker with Q-, G-, R-, or C-banding.

Q-banding was the first banding method described;[15,129] it uses the fluorescent dye quinacrine mustard or quinacrine dihydrochloride.

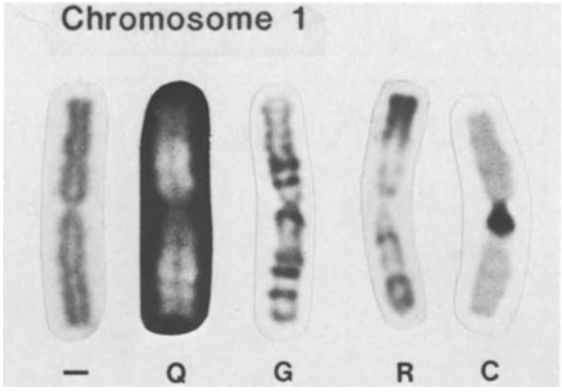


FIGURE 14–1 Chromosome 1 with various banding techniques. From left to right: Phase contrast, Q-banded, G-banded, R-banded, and C-banded. ×4000.

G-banding gives, with the exception of a few chromosome regions, a similar banding pattern as Q-banding and can be obtained by several pretreatment methods, followed by staining with Giemsa solution.[113] R-banding is obtained by pretreatment of the preparations with elevated temperatures, followed by staining with Giemsa. The banding pattern is the reverse of that obtained by Q- and G-banding.[25] C-banding is also produced by a modification of the pretreatment before staining with Giemsa; in human material it visualizes the centromeres only. This method can be especially valuable for the analysis of marker chromosomes with more than one centromere. C-banding must be combined with one of the other banding techniques for chromosome identification.[1] Many modifications of the different banding methods have been developed and usually each laboratory has its own routine (Figure 14–1).

Chromosome Nomenclature

Before specific chromosome abnormalities in lymphomas are discussed, a brief review of the nomenclature may be helpful. The nomenclature of human chromosomes has been standardized at several international conferences; the first was held in Denver, Colorado (1960), followed by conferences in London (1963), Chicago (1966), and Paris (1971).[91] Since 1971, a standing committee has taken the responsibility for necessary extension and modification of the nomenclature. The purpose of the meetings is to provide a system that is clear, simple, flexible, and easy to apply by the majority of cytogenetic laboratories.

In the recommended nomenclature, each pair of autosomes is serially numbered from 1 to 22 in descending order of length. The sex chromosomes are referred to as "X" and "Y." Autosomes of similar morphology can be divided into 7 recognizable groups from "A" to "G." The symbols "p" and "q" designate the short and long arm of each chromosome, respectively. A "+" or a "−" sign before the appropriate symbol means an additional or missing chromosome, respectively. A "+" or a "−" sign after the symbol means increase or decrease in length. For example, +7 means an extra chromosome 7 in the karyotype, 14q+ means a chromosome 14 with extra material on the long arm. The bands, produced by one of the different banding methods, are numbered consecutively from the centromere outward along each chromosome arm. The terms "distal" and "proximal" refer to the position of a band with respect to the centromere.

Each chromosome in the human somatic cell complement is defined as consisting of a continuous series of bands with no interbands. The bands are allocated to various regions along the chromosome arms and delimited by specific chromosome landmarks. In this system, a *landmark* is defined as a consistent and distinct morphologic feature that is an important diagnostic aid in identifying a chromosome. A *region* is defined as any area of a chromosome lying between two adjacent landmarks. Four items are required for the designation of a particular band: the chromosome number, the arm symbol, the region number, and the band number within that region. These items are given in order without spacing or punctuation. For example, 8q24 indicates chromosome 8, long arm, region 2, band 4.

Recently developed techniques for preparation of decondensed prometaphase chromosomes[127] made it necessary to extend the system for designation of chromosome regions and bands. Whenever an existing band is subdivided, a decimal point is placed after the original band designation, followed by the number of the subband. For example, the original band 8q24 can be subdivided into three subbands, designated 8q24.1, 8q24.2, and 8q24.3 (Figure 14–2).

The main types of structural aberrations are specified by the symbols "t" for translocation of chromosome material between chromosomes, "del" for loss of chromosome material, and "inv" for rotation of a chromosome segment 180°. The symbols are followed by parenthesis(s) indicating the chromosome(s) involved, e.g., del(15), inv(9). If chromosome material is translocated from one chromosome to another, the chromosomes involved are separated by a semicolon, e.g., t(8;14). In a second set of parantheses the regions and bands in which the breaks have occurred are specified. For example, t(8;14)(q24;q32) indicates a translocation between chromosomes 8 and 14; the break point in chromosome 8 is located in the long arm, region 2, band 4, and the break point in chromosome 14 is located in the long arm, region 3, band 2. A marker chromosome (mar) is a structurally abnormal chromosome. When the banding pattern is distinct enough to trace the origin of the chromosome material, this can be adequately described by the standard nomenclature. If only part of the marker can be identified, a question mark is used. For example, t(8;?) means a rearrangement in which only chromosome 8 could be recognized.

This "short system" for identification of structural abnormalities can be replaced by a "detailed system" which is a complementation of the first system. In addition to identifying the type of rearrangement, the detailed system defines each abnormal chromosome in terms of band composition. More information about chromosome nomenclature and the designation of structural abnormalities can be found in the reports of the 1971 Paris Conference[91] and the 1981 International System for Human Cytogenetic Nomenclature.[52]

Malignant Lymphomas

Chromosome studies on cells from lymphomas have been performed since 1962. The results obtained during the 1960s showed clearly that cells with an apparently normal diploid chromosome complement were interspersed with abnormal cells and that characteristic markers were associated with different types of lymphomas. A distinctive chromosome analysis was not possible, however, before banding methods had been introduced.

The first study on chromosomes from malignant lymphoma by banding techniques was performed by Fleischmann et al.,[29] who studied biopsies of seven tumors from lymph nodes with Q-banding. Marker chromosomes were noticed in four of them. The patients from whom the tumor material had been received had all undergone chemother-

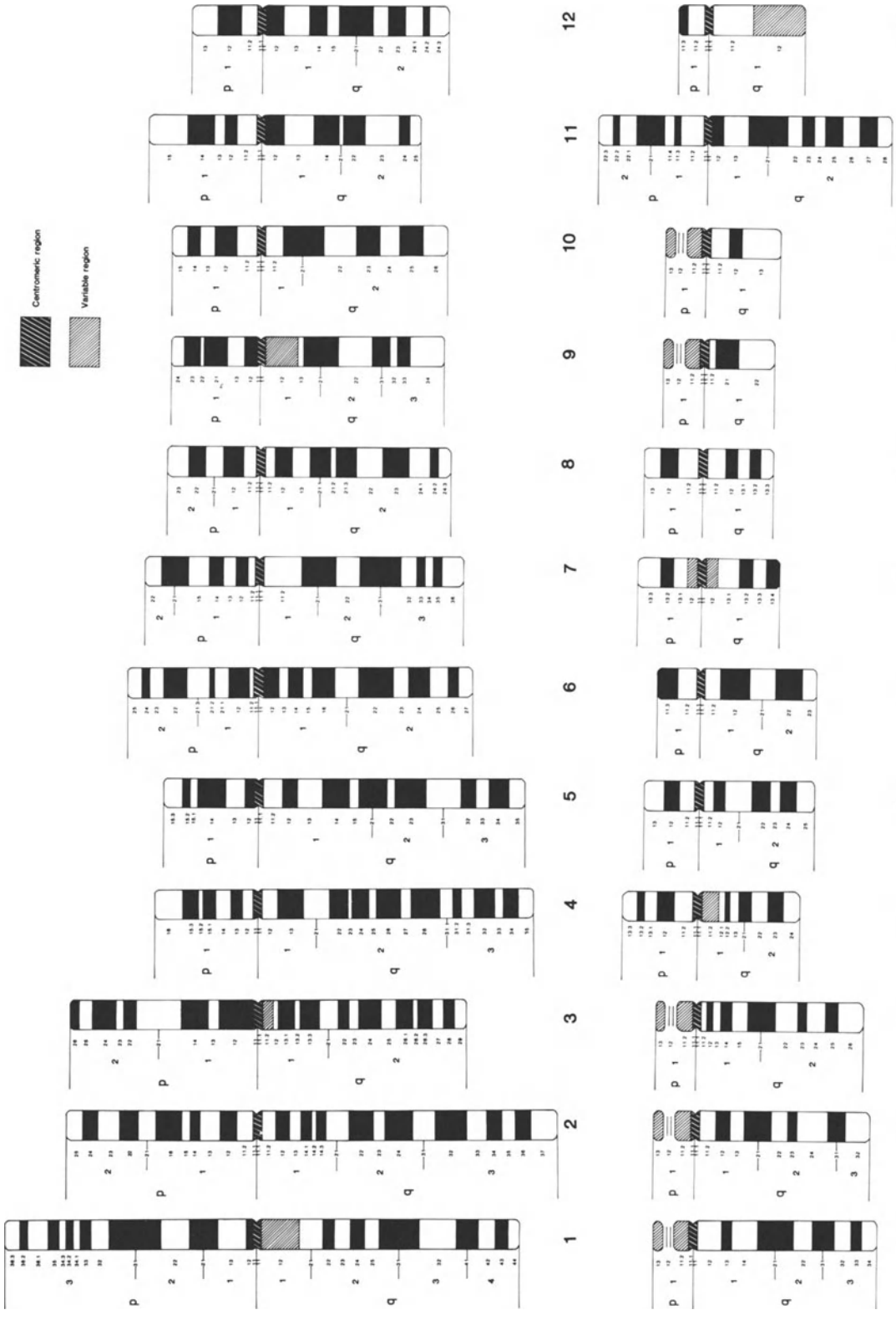


FIGURE 14–2 Diagrams of chromosome bands as observed with G-banding. This is a revised form of the diagram given in the Paris Nomenclature.[91] From ISCN, 1981,[52] with permission.

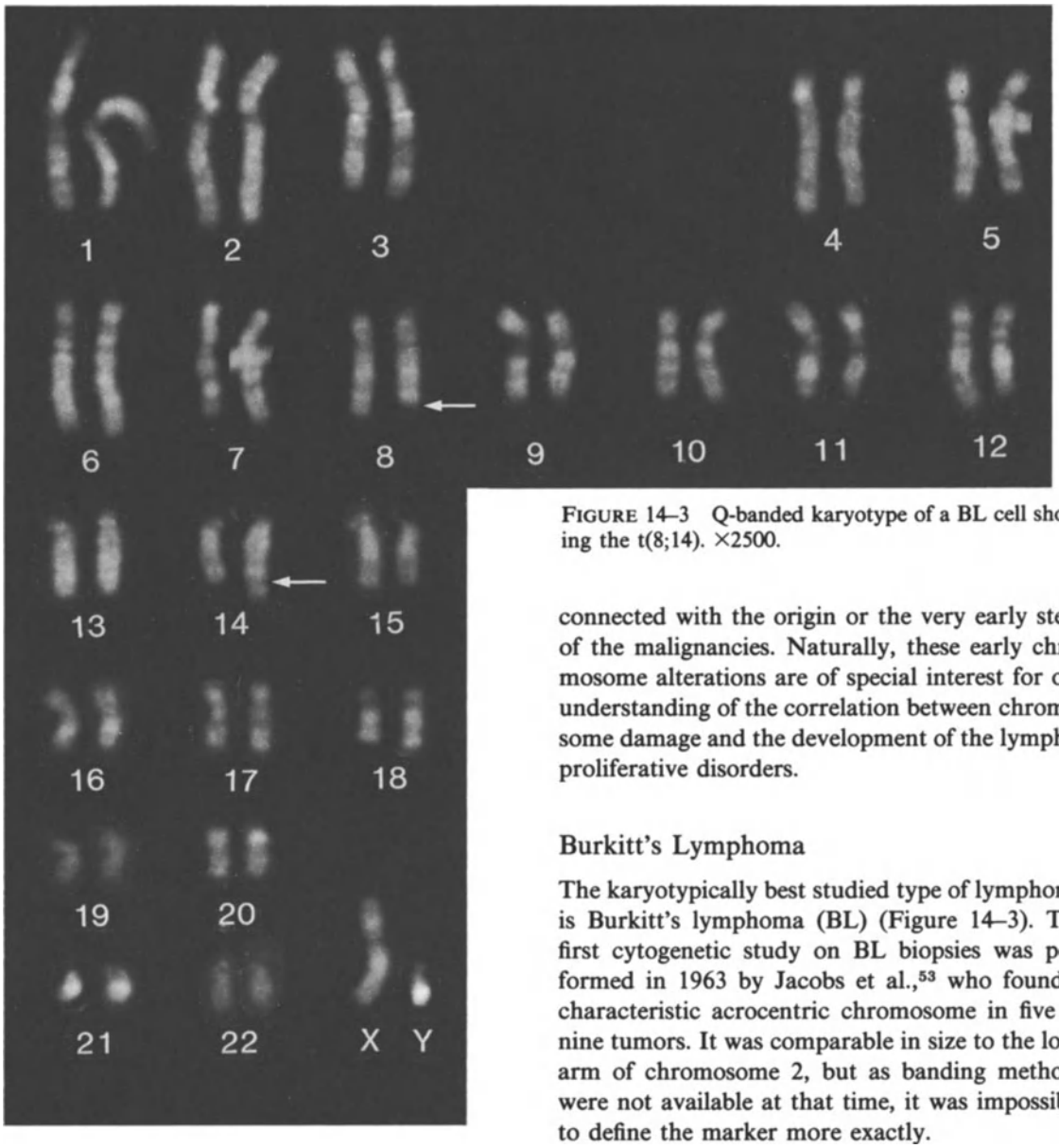


FIGURE 14–3 Q-banded karyotype of a BL cell showing the t(8;14). ×2500.

connected with the origin or the very early steps of the malignancies. Naturally, these early chromosome alterations are of special interest for our understanding of the correlation between chromosome damage and the development of the lymphoproliferative disorders.

Burkitt's Lymphoma

The karyotypically best studied type of lymphoma is Burkitt's lymphoma (BL) (Figure 14–3). The first cytogenetic study on BL biopsies was performed in 1963 by Jacobs et al.,[53] who found a characteristic acrocentric chromosome in five of nine tumors. It was comparable in size to the long arm of chromosome 2, but as banding methods were not available at that time, it was impossible to define the marker more exactly.

apy and radiotherapy, and therefore these first results from chromosome studies were regarded with some reservation.

At least 230 cases of malignant lymphomas have now been analyzed using banding techniques.[68,82,104] The accumulated results from these studies show nonrandom chromosome abnormalities confirming the findings of Fleischmann et al.[29] and suggesting that all types of lymphomas probably carry specific chromosome changes. Although many of the abnormalities described seem to be associated with tumor progression at later stages, a few marker chromosomes appear to be

Recurrent Chromosome Abnormalities

Translocation (8;14) In 1972 Manolov and Manolova reported results from African BL studied by G- and Q-banding methods.[71] The authors found an extra band at the end of the long arm of one chromosome 14 (14q+) in five of six tumor biopsies. The same abnormality was observed in seven of nine cell lines, established from biopsies from other patients. It soon became obvious that the 14q+ marker was the most consistent aberration found in Burkitt's tumors and in lines established from those tumors.[54,55,130] Zech et al.[130] ob-

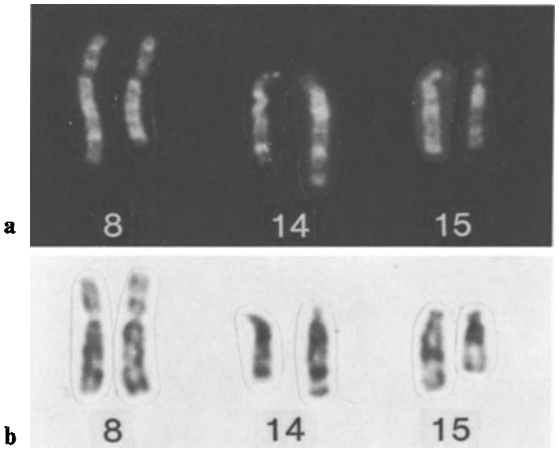


FIGURE 14–4 BL-translocation, t(8;14) (q24;q32). **(a)** Q-banding. **(b)** G-banding. ×2500.

served that cells from BL always possessed one chromosome 8 that was somewhat shorter than its homologue. Size and stainability of the missing segment corresponded to size and staining properties of the extra piece on 14q, and the authors concluded that the BL abnormality was a translocation between the long arms of chromosomes 8 and 14, t(8;14). The break point on chromosome 8 was localized to q24 and on chromosome 14 to q32. The translocation was thus designated as t(8;14)(q24;q32) (Figure 14–4).

In our study this translocation was found in eight of the BL biopsies and in two of four established BL cell lines but not in the peripheral blood of patients with BL (Figure 14–5). In two other BL biopsies and one BL cell line there was also a distinct 14q+, however, the technical quality of the metaphases was too poor to permit identification of the extra material on the chromosome.[130] Using methods for preparation of elongated prometaphase chromosomes from BL biopsies[131] and the BL-derived line Daudi,[73,133] we could identify the break points on the marker chromosomes 8 and 14 with still more precision to 8q24.13 and 14q32.33 (Figures 14–6 and 14–7). According to Manolova et al.[73] the t(8;14) is reciprocal.

Although further cytogenetic studies on BL confirmed the hypothesis that t(8;14) was the most consistent chromosome abnormality in cells from this malignancy, the question remained as to whether No. 8 or No. 14 should be regarded as the most characteristic marker of the disease. It was often suggested that chromosome 14 might be of crucial importance since it was noticed that metaphases of many non-Burkitt's lymphomas also possessed an extra segment on chromosome 14q, the origin of which could be traced to chromosome 8 but also to other chromosomes, such as Nos. 1, 4, 6, 10, 11, 13, 14, 15 and 20.[68,80,82]

Epstein-Barr Virus and the 14q+ Marker There seems to be enough evidence that the BL translocation is associated with lymphoid proliferation rather than with the Epstein-Barr virus (EBV) infection. This suggestion is supported by the fact that EBV genome–negative Burkitt's tumors, and cell lines derived from such tumors, do contain a 14q+ chromosome[55,132] (Figure 14–5). It is in line with these observations that the EBV genome–negative line BJAB, which does not possess the marker, could be converted into the EBV genome–positive subline GC-BJAB without any visible change of the two normal chromosomes 14.[132]

Variant Translocations Recent findings of BL with variant translocations have changed our concept of the role of the chromosomes involved in the disorder. At least 14 cases have been described in which a chromosome 8q− is present but the recipient chromosome for the deleted segment is not a 14q. The first 2 patients belonging to this group were reported from Belgium[120] and Japan,[84] and their tumor cells had identical translocations

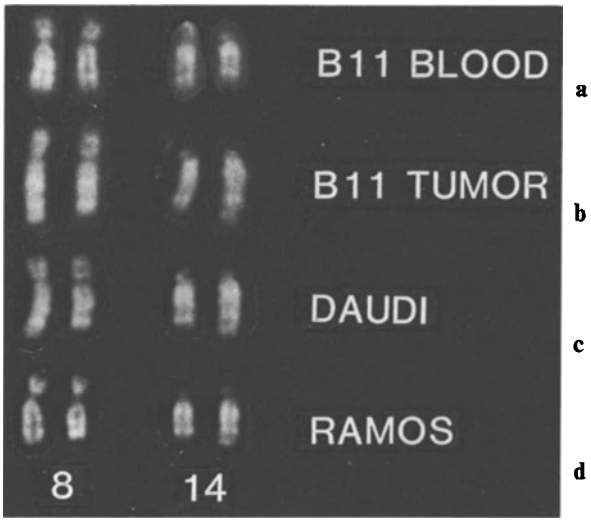


FIGURE 14–5 Chromosomes 8 and 14 in **(a)** blood and **(b)** tumor of a patient with Burkitt's lymphoma, **(c)** the EBV genome positive BL line Daudi and **(d)** the EBV genome negative BL line Ramos. Q-banding. ×2500.

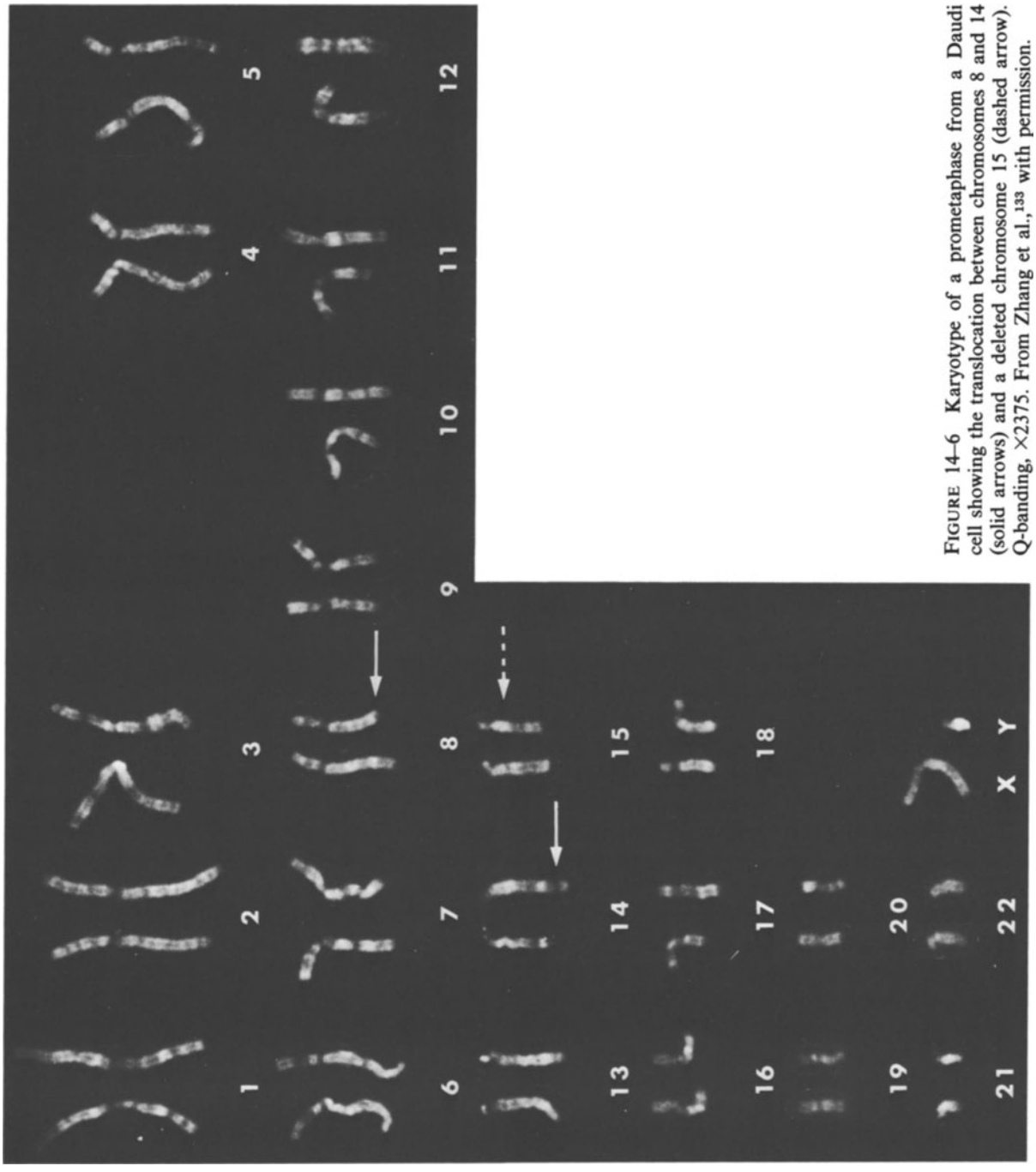


FIGURE 14–6 Karyotype of a prometaphase from a Daudi cell showing the translocation between chromosomes 8 and 14 (solid arrows) and a deleted chromosome 15 (dashed arrow). Q-banding, ×2375. From Zhang et al.,[133] with permission.

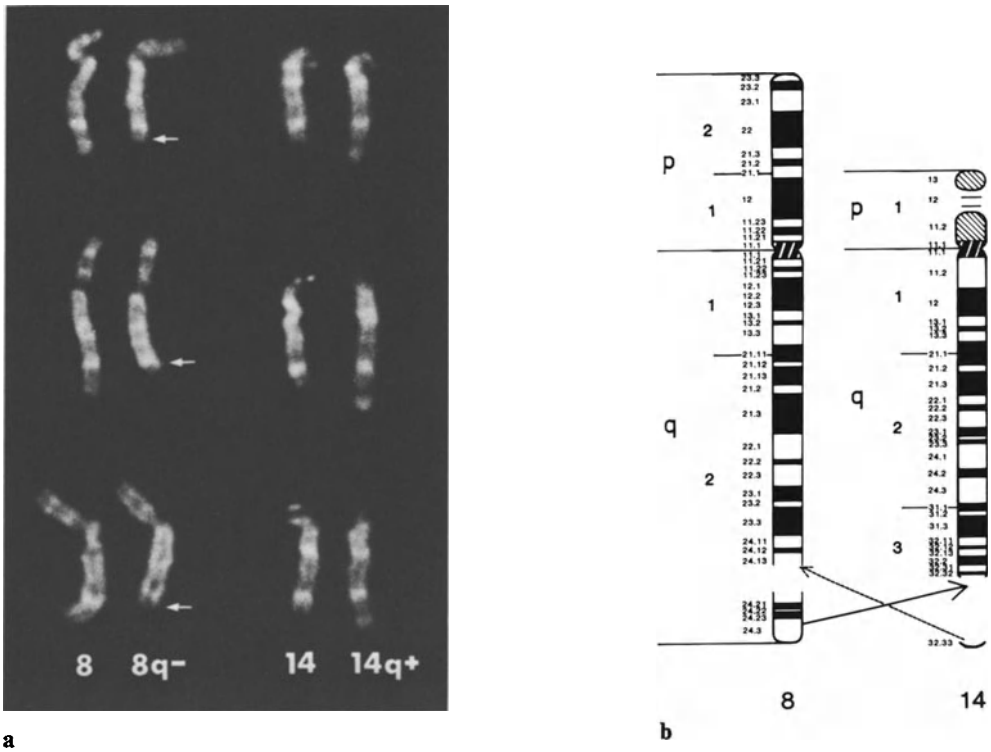


FIGURE 14–7 BL translocation, t(8;14) (q24.13;q32.33). **(a)** Karyotype of a prometaphase. Q-banding, ×2500. **(b)** Diagram of the translocation showing the break points in the decondensed chromosomes.

between chromosomes 2 and 8 as the sole abnormality. The break points were localized in 2p12 and 8q23 or 8q24 (Figure 14–8). The same type of translocation was also found in BL from other countries, including the United States.[9,11,55] One patient with acute lymphoblastic leukemia (ALL) whose cells had L_3 (Burkitt-type) morphology was recently reported by Rowley et al.;[105] this report was of special interest because it provided additional evidence for a close relationship between karyotypic changes in BL and Burkitt-type ALL.

Berger[7] was the first author who detected a variant translocation between chromosomes 8 and 22 with break points in 8q24 and 22q11 (Figure 14–9). More translocations of this type have now been published.[9,10,31,117] In chronic myelocytic leukemia several cases with translocations involving three chromosomes have been described. This type of complex translocation also exists in BL, as reported by Philip et al.[94] The different types of variant translocations described to date are summarized in Table 14–1.

A few cases of BL and ALL L_3 without a t(8;14) or even without any detectable chromosome rearrangements at all have been published recently.[24,110] Unfortunately, it is at present not possible to distinguish between normal and malignant metaphases if the karyotype appears normal; more studies are necessary before those results can be interpreted. It is not clear yet whether the clinical course of the disease is dependent on the type of translocation; the answer to this question needs further investigation.

Burkitt's Lymphoma–Derived Cell Lines

BL lines are of great importance for many types of work, such as immunologic studies or somatic cell hybridization, and therefore warrant description. BL cells are fast growing, easy to maintain in culture, and therefore easy to establish. Nevertheless, different lines differ remarkably from each other with respect to karyotypic stability and their tendency for polyclonal development.

A line that is still very stable after more than 14 years is the Daudi line, originating from a BL of an 8-year-old boy and established in 1968.[60] In our laboratory the majority of cells from this line exhibit only two consistent aberrations, the

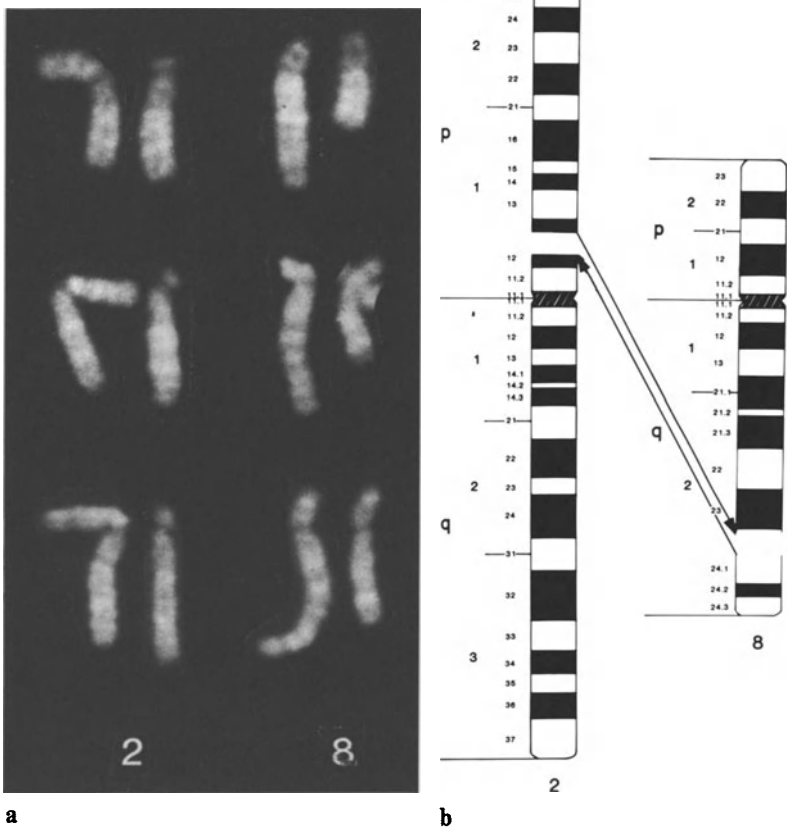


FIGURE 14–8 Variant BL translocation, t(2;8) (p12;q24). **(a)** Partial karyotypes. Q-banding, ×2500. **(b)** Diagram of the translocation showing the break points.

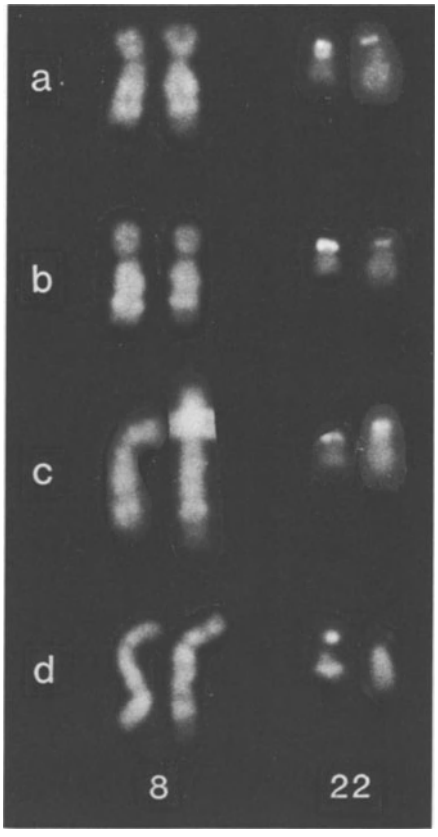
characteristic BL translocation, t(8;14), and an interstitial deletion of the long arm of chromosome 15, del(15) (Figures 14–6 and 14–10). Occasionally other chromosome abnormalities, especially +7, can be observed. Since Daudi cells are unable to produce β_2-microglobulin, and the gene locus for this protein has been assigned to chromosome 15,[47] several authors have suggested that the deleted segment carries the exact locus for the gene.[72,130,132,133]

Hodgkin's Disease

In adults, Hodgkin's disease (HD) is the most common type of malignant lymphoma and as common as leukemia. The number of karyotyped cases of HD is nevertheless relatively low (41 cases). This may be explained by incomplete histopathologic staging of the disease in many cases studied. In the great majority of cases, chromosome studies of malignant cells from patients with HD have been performed on lymph nodes. Effusions were often considered as being unsuitable because cytologic and histologic examination of pleural or peritoneal exudates yielded cells that appeared uncharacteristic for the disease.[58] However, it now seems clear that effusions contain cells with characteristic abnormalities and therefore are worthy of analysis.[50]

The most characteristic karyotypic feature for HD is the considerable proportion of normal cells, as much as 70%–90%. Among abnormal cells, extensive numerical aberrations, in most cases extra chromosomes, are noticed. Often as much as 50% of the abnormal metaphases are in the triploid or in the tetraploid range.[32,33,50,96] All chromosome groups have been involved in the aberration and no specific abnormality could be identified. The same is true of structural changes. Abnormal metaphases usually contain a number of rearrangements rather than a few specific markers. Perhaps a 14q+ chromosome can be regarded as one of the more characteristic markers for HD, as it is for other lymphoproliferative diseases.[17,32,33,50,83] In some cases the 14q segment has been traced to 8q, but other donor chromosomes have been found in the majority of cases.

Usually the break point on chromosome 14 is in 14q32, as in BL, however, a break in 14q13



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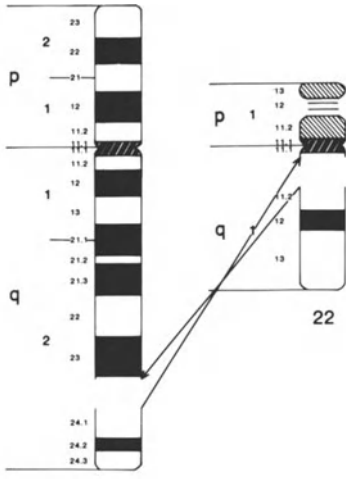


FIGURE 14–9 Variant BL translocation, t(8;22) (q24;q11). **(a)** Partial karyotypes. Q-banding, ×2500. **(b)** Diagram of the translocation showing the break points.

was found by Lawler et al.[66] in one of eight patients with HD (Table 14–2). All patients had been treated by chemotherapy and total nodal irradiation. A break in 14q13 was also found by Fukuhara et al.[36] in an HD line derived from pleural effusion cells of a patient with HD, and in cells from bone marrow and nonstimulated blood of three patients with non-Hodgkin's lymphomas. These cases seem noteworthy because they may indicate a second vulnerable region on 14q in q1.

The complexity of karyotypic changes in HD requires further studies on well-defined histopathologic cases. There has been a good deal of discussion about the perplexing finding that such a remarkably large number of metaphases from HD are normal; it has been suggested that improved cytogenetic methods might reveal more abnormalities than have been observed to date.[107]

Non-Hodgkin's and Non-Burkitt's Lymphomas

Other types of malignant lymphomas, including poorly, moderately, and well-differentiated lymphoma, lymphosarcoma, and diffuse histiocytic lymphoma, are often classified incompletely or according to different nomenclature systems. Therefore, it is perhaps premature to discuss the chromosome condition of each group separately, even though the detection of specific markers would be helpful in distinguishing the different types and stages of the disorders. In the majority of studies, lymph nodes or material from extranodal sites have been used for the investigations. In a few cases bone marrow and peripheral blood have also been analyzed. The general result of the studies has been the presence of at least one malignant cell clone with complex karyotypic rearrangements.[28-30,32-36,56,62,65,68,74-77,96,97,104]

Recurrent Chromosome Abnormalities

In spite of the complex and often confusing karyotypic picture in the malignant cells, the following chromosomes are nonrandomly involved in aberrations:

Chromosome 14 as 14q+
Chromosome 6 as 6p− or 6q−
Chromosome 1 as 1p−, 1q−, or rearranged
Chromosome 11 as 11q−
Chromosomes 3, 7, and 8 as extra chromosomes
Chromosomes 6, 15, 20, and 22 as missing chromosomes.

Some of these changes are of special interest because they seem to be of major importance for B cell malignancies in general.

TABLE 14–1 Variant Translocations in Burkitt's Lymphoma

Type of Translocation	Geographic Origin	Tumor Site	Diagnosis	EBV Genome Present	Preparation Source	Sex	Reference
t(2;8)(p12;q23)	Belgium[a]	Maxilla	BL	+	Lymph node	F	van den Berghe et al.[120]
t(2;8)(p12;q24)	Japan	Ascites lymphoma	BL	+	Ascites, marrow, established line	M	Miyoshi et al.[84]
t(2;8)(p12;q24)	Germany[a]	Several organs	BL	+	Cell line	F	Bornkam et al.[11]
t(2;8)(p12;q24)	Kenya	Maxilla	BL	+	Established line (LY66)	M	Bernheim et al.[9]
t(2;8)(p12;q24)	Uganda	Ovary	BL	+	Established line (LY91)	F	Bernheim et al.[9]
t(2;8)(p13;q24)	United States	Bone marrow	All 13 (Burkitt type)	—	Bone marrow, peripheral blood	M	Rowley et al.[105]
t(8;22)(q24;q11)	Turkey, France[a b]	Ascites, endema in both legs	BL + ALL	—	Blood (without PHA) bone marrow, ascites	M	Berger et al.[7]
t(8;22)(q23;q12)	France[a]	Maxilla	BL	—	Bone marrow, cell line	M	Bertrand et al.[10]
t(8;22)(q23;q11)	France[a]	Cervical region	BL	+	Tumor biopsy	M	Fraisse et al.[31]
t(8;22)(q24;q11)	France[a]		BL	—		M	Tanzer et al. 1980[117]
t(8;22)(q24;q11)	Africa	Lymph node	BL	+	Established line (MAKU)	M	Zech et al. 1976[130]
t(8;22)(q24;q11)	Uganda		BL	+	Established line (Ly47)	M	Bernheim et al. 1981[9]
t(8;22)(q24;q11)	Kenya	Recurrence	BL	+	Established line (LY67)	F	Bernheim et al.[9]
t ins[d] (2;8;9)[c]	Alger	Bone marrow	BL	+	Bone marrow, established line	M	Philip et al. 1982[94]

[a] Caucasian.
[b] Lived in France since 1951.
[c] Complex rearrangement with translocation and insertion.
[d] A segment of another chromosome is inserted.

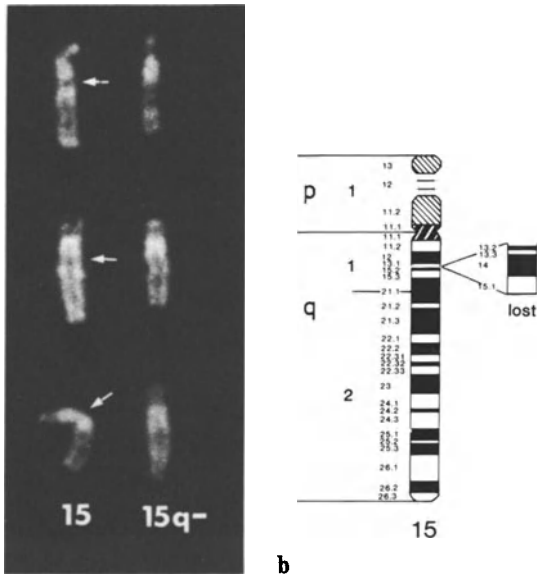


FIGURE 14–10 Decondensed chromosome 15 of Daudi cells. **(a)** Partial karyotypes of normal chromosome 15 and deleted chromosome 15. The arrows indicate the segment missing in the deleted chromosome. Q-banding, ×2500. From Zhang et al.,[133] with permission. **(b)** Diagram of decondensed chromosome del(15).

Chromosome 14q+ This marker is probably the most characteristic abnormality for B cells in general, even though it has also been noticed in cells of T origin, as mycosis fungoides, chronic T cell leukemia, one T cell line (derived from ALL), in neurogenic tumors[49,126] and a clone of stimulated lymphocytes in ataxia gelangiectasia.[34,35] With a few exceptions,[28,36] the break point is in q32, supporting the hypothesis that a specific property of the chromosome, localized in this vulnerable region, has to do with the disorder.

The segment translocated to the long arm of chromosome 14 can be of different origins; it is especially often from chromosome 8,[68] but it seems that most chromosome types may be engaged as donors.[77,80] Chromosomes 1, 11 and 18 are more often involved than others,[32] and it is obvious that the donor chromosome in non-BL is not as constant as in BL.

Chromosome 6q−, apparently even more common than the 14q+, may also be of significance, even though we do not understand its function.[56,68,77] It has a widespread incidence and has also been found frequently in ALL,[118] chronic lymphocyte leukemia (CLL),[37] myeloproliferative diseases, carcinomas, malignant melanomas, and neurogenic tumors.[82] The break points on 6q are apparently varying and preferentially localized between 6q13 and 6q22 or 6q25. In a few cases, a deletion of 6p has been observed, although this abnormality is not as common as the 6q aberration.

Other Abnormalities. Chromosome 1 rearrangements Fukuhara et al. drew attention to a very interesting correlation between the occurrence of a 14q+ chromosome and structural abnormality of chromosome 1, observed in 11 patients with diffuse histiocytic lymphoma, while only 2 patients with abnormal No. 1 were found among 11 patients with normal No. 14.[34] In another group of patients with poorly differentiated lymphoma, 5 of 9 had a 14q+ and a No. 1 abnormality.[34] Correlations between a 14q+ and a No. 1 abnormality have been found in other studies of lymphomas

TABLE 14–2 Frequency of Involvement of Chromosome 14 in 25 Cases of Hodgkin's Disease

No. of Cases	Numerical Aberrations of No. 14	Structural Aberrations of No. 14	Break Point on No. 14	Reference
6	—	3	14q32	Fukuhara et al.[33] [a]
1	—	1	14q32	Zech et al.[130]
2	1	—	—	Cavallin-Ståhl et al.[17]
6	1	2	14q32	Hossfeld and Schmidt[50] [b]
1 (line)	—	1	14q13	Fukuhara et al.[36]
7 (blood)	2	2	14q1 (1 case) 14q32 (1 case)	Lawler et al.[66]
1 (marrow)	—	1	cen (14)	Lawler et al.[66]

[a] One case, a Hodgkin's-derived line, was reinvestigated and reinterpreted as having a break in 14q24 or 32.

[b] One patient had both a +14 and a 14q+.

as well;[96,97,115] these observations warrant further studies to clarify the correlation.

The problem is of special interest because of the frequency with which chromosome 1 is involved in other malignancies, such as cervical carcinoma,[3,4] breast tumors,[23] lung tumors,[131] neurogenic tumors,[74] bladder carcinomas,[2] and ovarian carcinomas.[5] It seems likely that there do exist additional cases with a 14q+ abnormality plus a structurally abnormal No. 1 in aberrant metaphases of some lymphomas, even though they are easily overlooked in highly aberrant cell clones.

Mark et al. point out that special attention should also be given to chromosome 3 because a +3 chromosome was the sole aberration in one lymphoma they studied, and in another case +3 and −8 were the only abnormalities.[77] Chromosome 3 was also frequently involved in structural rearrangements, with the break points concentrated at the centromeres and regions that stain lightly with Q- and G-banding methods.[76]

Diagnostic and Prognostic Significance of Marker Chromosomes

As mentioned above, it is at present very difficult to correlate certain chromosome aberrations with types of lymphomas because different classification systems are used by different authors. Nevertheless, a few studies on larger groups of patients have indicated that the 14q+ marker may be more common in poorly differentiated lymphoma (15 of 35 cases) than in diffuse histiocytic lymphoms (15 of 32 cases).[34,35] Fukuhara et al. were also able to correlate a 14q+ abnormality with morphology, i.e., with a large noncleaved nucleus in the malignant cells.[34] Thus, there is good reason to expect that in the future lymphomas may be characterized in terms of the nature of their chromosome 14 morphology, although more studies are necessary before this will be possible.

T Zone Lymphomas

The T zone lymphoma, a type of non-Hodgkin's lymphoma, was first described by Lennert[67] as a nonleukemic type of lymphoma containing all the components of T regions of lymphoid tissue. Only a few patients with this disorder have been karyotyped.

In one study of five patients,[44] the karyotyped metaphases of one patient showed no abnormalities, although the possibility could not be excluded that cells with abnormal karyotype had not divided. The other four patients exhibited highly abnormal cells. Nevertheless, the overrepresentation of certain abnormalities seemed clear. Chromosome 3 was often involved either numerically (+3) or structurally. A chromosome 14q+ occurred in only one of the five patients of this series; this patient had two different 14q+ markers—one was a Y;14 translocation and the other a t(14;?). Two different chromosome 14 markers are extremely rare events, previously described only by Catovsky et al.[16] in a follicular lymphoma, by Fukuhara and Rowley[32] in a malignant lymphoma of mixed cell type, and by Reeves and Pickup[97] in a centroblastic–centrocytic lymphoma.

Acute Lymphoblastic Leukemia

In the report of the Third International Workshop on Chromosomes in Leukemia[118] the cytogenetic results of studies on 346 patients with ALL are presented. Of these patients, 112 were karyotypically normal, and chromosome changes had occurred in 234. The study of preparations of decondensed prometaphase chromosomes[127] will probably reveal abnormalities in more patients[128] even though these chromosomes are extremely difficult to analyze in cells that contain complex rearrangements.

Recurrent Chromosome Abnormalities

Numerical Changes Both treated and untreated patients in the Third International Workshop study had numerical chromosome changes, and these changes occurred along with structural changes in 113 of 234 cases (Table 14–3). The chromosomes involved in the numerical changes could not always be identified, mainly because of technical problems with cells having more than 50 chromosomes. Therefore, those cells were often excluded from banding analysis. Even though these shortcomings must be taken into consideration, it seems clear that chromosomes 6, 8, 18, and 21 were overrepresented, and chromosomes 1, 2, and 3 were underrepresented. Chromosomes 7 and 20 were more often lost from metaphases than others.

Structural Changes The frequency of the most common types of structural rearrangements is summarized in Table 14–4. The most predominant

TABLE 14–3 Frequency of Numerical Chromosome Aberrations in Patients with ALL

Aberration	Patients Untreated	Patients Treated	Patients with Numerical and Structural Changes	Total
Hyperdiploid				
47–50 chromosomes	28	—	12	40
>50 chromosomes	31	—	12	43
Pseudodiploid	41	2	81	124
Hypodiploid	17	3	8	28

From the Third International Workshop on Chromosomes in Leukemia,[118] with permission.

chromosome abnormality was a 22q− marker, which was found in 45 cases (39 untreated). In the majority of cases the 22q− was identified as a classical Ph[1] translocation, t(9;22)(q22;q11). Two cases had variant translocations, t(6;22)(p25;q11) and t(21;22)(q22;q11). One complex translocation was found involving three chromosomes, t(3;9;22). In one case the lost segment of 22q could not be detected. About one-half of the patients showed abnormalities in addition to the Ph[1] chromosome. These abnormalities were both numerical and structural. Most common was a gain of a second Ph[1] chromosome or an extra chromosome 8.[118]

A t(8;14) was found in 17 patients (1 untreated). The break points were identical in all cases, i.e., 8q24 and 14q32. There was an excess of males compared to females and of adults compared to children in this group. A 14q+ was found in 15 patients, all untreated. In 6 patients the 14q+ was due to a balanced translocation, involving chromosome 11 in 4 patients. This group also had an excess of males compared to females and adults compared to children.[101,114,118]

Two other types of translocations were clearly nonrandom events. A t(4;11) was seen in 19 patients (18 untreated) and a 6q− occurred in 16 patients (13 untreated). Five patients had the 6q− as the sole abnormality.[118]

ALL with Near-Haploid Clones Kaneko and Sakurai described a 17-year-old Japanese female ALL patient with a clone characterized by a haploid chromosome number plus an additional chromosome 6, 10, 18, and X.[57] Another clone had 56 chromosomes, i.e., about twice the number of the near-haploid cells. The authors drew attention to two other patients[59,90] who showed a similar, near-haploid karyotype with few additional chromosomes. Furthermore, a patient of Prieto et al., although studied without banding methods, seemed to belong to this group of patients because he had a clone with haploid karyotype plus an extra D and an extra G chromosome, the latter most likely a 21.[95] The chromosomes present in two copies in the near-haploid clones of the different patients are listed in Table 14–5. All patients belonging to this group also had a clone with a

TABLE 14–4 Frequency of Nonrandomly Distributed Structural Chromosome Aberrations in 112 Patients with ALL

Aberration	Patients Untreated	Patients Treated	Total
Ph[1]	39	6	45
t(4;11)	18	1	19
t(8;14) and other t(8)	16	1	17
14q+[a]	15	—	15
6q−	13	3	16

From the Third International Workshop on Chromosomes in Leukemia,[118] with permission.
[a] One t(8;14)(q21;q24) and four t(11;14).

TABLE 14–5 Chromosomes Present in Two Copies in Near-Haploid Clones of ALL

Chromosomes[a]										References
			10		18				X	Kessous et al.[59]
			10	14	18					Oshimura et al.[90]
	6		10		18				X	Kaneko and Sakurai[57]
1	6	9	10		18	19	21	22		Shabtai and Halbrecht[110]
				D			G			Prieto et al.[95]

[a] All banded except chromosones D and G in report by Prieto et al.

double number of chromosomes. The similar course of the disease in three patients[57,59,90] suggests that a special hyperhaploid subclone was directly correlated with a special subgroup of ALL.[57]

Clinical Significance of Cytogenetic Findings

The accumulated clinical and cytogenetic data collected and analyzed by The Third International Workshop on Chromosomes in Leukemia[118] allowed, for the first time, the correlation of specific karyotypic changes with prognosis. The outcome of the analyses was that patients with a modal chromosome number over 50 responded best to treatment. Children in this group often appeared to be cured. Patients without karyotypic changes or with a 6q− also had a good prognosis. In contrast, patients with a t(4;11) or t(8;14) responded poorly and had shorter survival.

Patients with a Ph[1] chromosome also had a poor prognosis. The survival was 2–24 months, with a median for both children and adults of about 12 months. This contrasts with a median survival of more than 2 years in adult Ph[1]-negative ALL.[19,103] The collection and analysis of more cases will probably provide more detailed information. Although further studies are necessary, the data provided by the Workshop indicate that cytogenetic features are an important prognostic factor in ALL.

Chronic Lymphocytic Leukemia

In approximately 90% of cases, CLL is a B cell disorder. The karyotypic picture was poorly understood in the past because in PHA-stimulated blood cultures it is mainly the nonneoplastic T cells that proliferate, not the neoplastic B cells. The number of spontaneously dividing malignant cells is low in lymph nodes and marrow, and cytogenetic data were only available from a few cases studied in late phase, when immature dividing lymphocytes were circulating in the blood. Due to the low number of adequate metaphases examined, it was generally assumed that chromosome abnormalities were sporadic and random events in B cell CLL.[22] Exceptions, such as t(8;14), t(11;17), t(1;10), and del(11), described in four cases by Fleischman and Prigonina,[28] were discussed with reservation because of the difficulties mentioned above.

The discovery of mitogens that primarily stimulate B cells, the polyclonal B cell activators, opened new possibilities for the cytogenetic investigation of CLL. It is now possible to induce DNA synthesis, cell division, and monoclonal immunoglobulin synthesis in peripheral blood from patients with B cell CLL.[39–41,98]

Recurrent Chromosome Abnormalities

The total number of cases studied so far is at least 134 (Table 14–6). In the studies of Hurley et al.[51] and Najfeld et al.[87] cell lines were used, and in the report by Autio et al.[6] the number of analyzed metaphases was very low. These three reports are therefore difficult to evaluate. In the remaining publications, 57 patients were studied, 37 (62%) of whom showed clonal abnormalities. The aberrations were both numerical and structural (Table 14–7). Chromosomes 12, 3, 17, and 20 were most often involved numerically and chromosomes 14, 17, 6, and 22 structurally.[37,39–42,86]

Trisomy 12 This was the most common abnormality (Figure 14–11), found in 19 of the 37 patients. One patient had only partial trisomy of 12q (Figure 14–12).[38] In 6 patients an extra chromosome 12 was the only abnormality, while in the other 13 patients additional aberrations were seen. Other trisomies (+3, +17) occurred, often together with trisomy 12. Chromosome 17 was also in-

TABLE 14–6 Clonal Chromosomal Aberrations in 134 Patients with B Cell CLL

Patients Studied	Patients with "Adequate" No. of Metaphases	Patients with Clonal Aberrations	Patients with +12	References
22	14	11	10[a]	Gahrton et al.[39-41] Gahrton and Robèrt[37] Robèrt et al.[99]
2[b]	2	2	1	Hurley et al.[51]
12	12	5	—	Gahrton and Robèrt,[37] Nowell et al.[88]
2[b]	2	2	1	Najfeld et al.[87]
27	19	9	5	Morita et al.[86]
66	>5	5	2	Autio et al.[6] Schröder et al.[109]
Total	>57	37	19	

[a] One case with patial trisomy 12q13 → qter.
[b] Cell cultures.

volved in monosomy, as well as chromosomes 20 and 3.

The fact that trisomy 12 was the only aberration in 6 patients may indicate that the +12 in these cases was the first step in the development of a malignant clone and that other chromosome aberrations were secondary changes. One may ask whether trisomy of the whole chromosome 12 is necessary for development of the malignancy or whether there is a specific segment that is critical for the disease. The patient of Gahrton et al. with partial trisomy of chromosome 12 had this abnor-

TABLE 14–7 Abnormal Karyotypes in CLL Patients with Extra Chromosome 12 with or Without Additional Aberrations

Karyotpye	No. of Patients	References
47,XY,+12	4 + 2	Gahrton et al.[40,41] Morita et al.[86] Schröder et al.[109]
47,XX,+12	3	Gahrton et al.[40,41] Hurley et al.[51] Najfeld et al.[87]
46,XY,del(11)(q22),+12,−13,−21, mar(21;?)−22,+2mar/45,XY,del(11) q22),+12,−13,−15,mar(15;?),−21, mar(21;?),−22/46,XY,del(11)(q22), +12,+12,−13,−21,−22,+2mar/46,XY, t(1;8)(p22;qter)	1	Gahrton et al.[41]
47,XX,+12,del(14)(q24)	2	Gahrton et al.[41] Schröder[109a]
47,XX,+12,del(2)(q31)	1	Gahrton et al.[41]
46,XY,+12,−17/47,XY,+12,−17,+mar	1	Robèrt et al.[99]
46,XY,+12,−20/45,XY,−20	1	Robèrt et al.[99]
47,XY,22q−,+12	1	Morita et al.[86]
48,XX,+3,+12/47,XX,+12/46,XX, −11,+12/45,XX,−11	1	Morita et al.[86]
47,XY,−20,+3,+12/47,XY,−20,+9, +12/46,XY,−20,+12/45,XY,−20	1	Morita et al.[86]
46,XY,t(10;10)(pter;q24), dup(12)(q13→22)	1	Gahrton et al.[38]

[a] Break point not noted by Schröder.

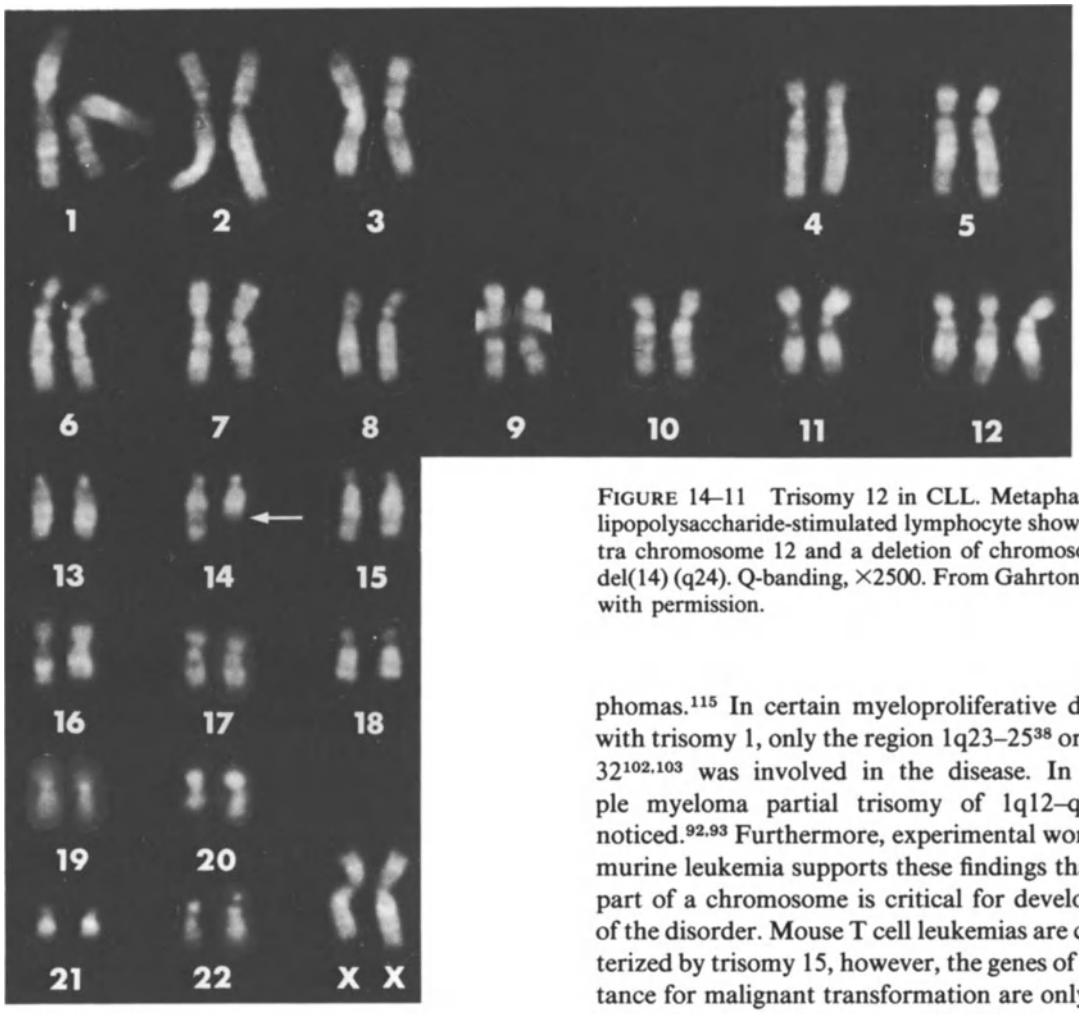


FIGURE 14–11 Trisomy 12 in CLL. Metaphase from lipopolysaccharide-stimulated lymphocyte shows an extra chromosome 12 and a deletion of chromosome 14, del(14) (q24). Q-banding, ×2500. From Gahrton et al.,[41] with permission.

mality in 13 of 63 metaphases.[42] The duplication involved the region 12q13 → qter, i.e., the trisomy was limited to this short segment of the long arm (Figure 14–12). The authors concluded that duplication of the genes located in 12q13 → 12qter was of crucial importance for the development of the disorder. It seems of interest that Miyoshi et al. described an adult T-cell leukemia with a rearrangement of chromosome 12.[85] The break point was localized to q13, i.e., the same region that showed the break in the case of the partial trisomy 12.[42] This could mean that 12q13 is a vulnerable region and easily affected by DNA-damaging agents.

Several other cases are known in which partial trisomy of a chromosome can be correlated with malignancy. A duplication of part of the long arm of chromosome 1, between bands 1q25 and 1q32, was found in two cases of lymphocytic lymphomas.[115] In certain myeloproliferative diseases with trisomy 1, only the region 1q23–25[38] or 1q25–32[102,103] was involved in the disease. In multiple myeloma partial trisomy of 1q12–q3 was noticed.[92,93] Furthermore, experimental work with murine leukemia supports these findings that only part of a chromosome is critical for development of the disorder. Mouse T cell leukemias are characterized by trisomy 15, however, the genes of importance for malignant transformation are only those located distal on chromosome 15 (distal to T6).[122]

Chromosome 14 Structural aberrations involving chromosome 14 were of special interest. Deletion of 14q was found in two patients, and one patient had a translocation between chromosomes 11 and 14 (Figure 14–13).[37] The most common aberration of chromosome 14 was a 14q+, found in 10 patients.[39,41,86,88,109] The donor chromosome for the extra segment on 14 could not always be determined, but in three patients it was shown to be 11q.

Chromosome 6 This chromosome was also involved in several structural aberrations. In the majority of cases 6q was deleted, but a 6p− was also seen in some patients.[37]

Ph¹ Chromosome A Ph¹-like chromosome has been described in a few patients with CLL. In one patient, reported by Morita et al., the short chromosome 22 was designated as del(22)(pter-

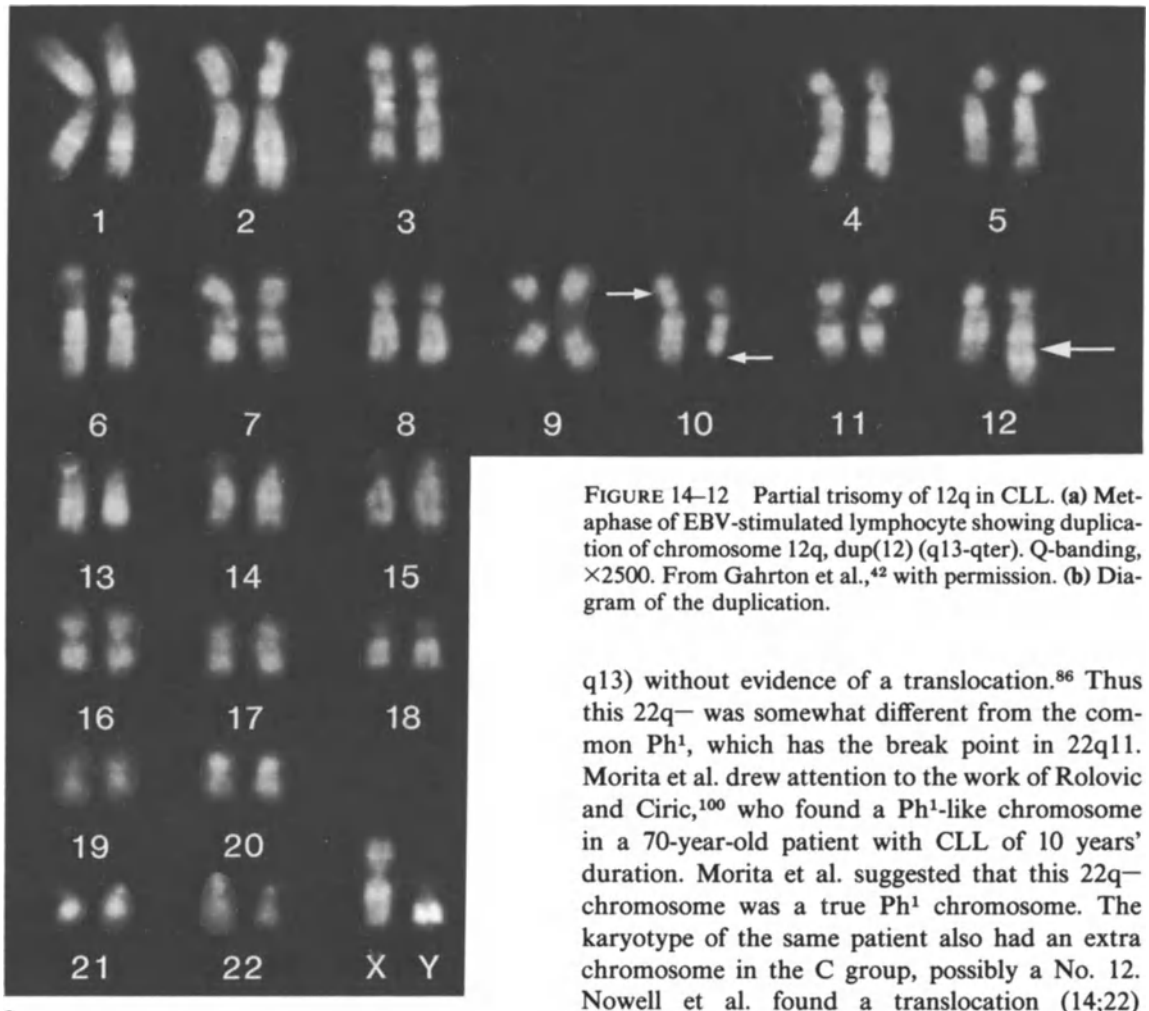
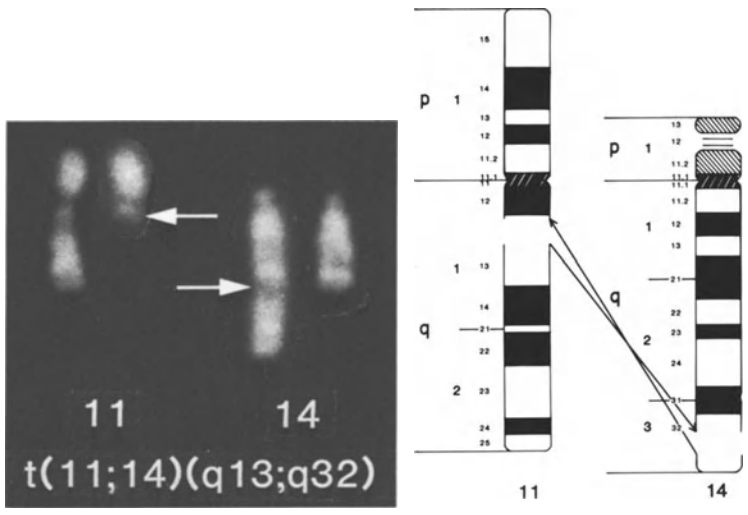


FIGURE 14–12 Partial trisomy of 12q in CLL. **(a)** Metaphase of EBV-stimulated lymphocyte showing duplication of chromosome 12q, dup(12) (q13-qter). Q-banding, ×2500. From Gahrton et al.,[42] with permission. **(b)** Diagram of the duplication.

q13) without evidence of a translocation.[86] Thus this 22q− was somewhat different from the common Ph¹, which has the break point in 22q11. Morita et al. drew attention to the work of Rolovic and Ciric,[100] who found a Ph¹-like chromosome in a 70-year-old patient with CLL of 10 years' duration. Morita et al. suggested that this 22q− chromosome was a true Ph¹ chromosome. The karyotype of the same patient also had an extra chromosome in the C group, possibly a No. 12. Nowell et al. found a translocation (14;22) (q32;q11) in a patient with CLL.[88] Thus this case had not only a 14q+ but a Ph¹ also. According to Morita et al.,[86] these examples strengthen the hypothesis that a Ph¹ chromosome may occur in cells of CLL.

EBV Stimulation and Chromosome Aberrations
Morita et al. also investigated EBV-stimulated lymphocytes from five normal individuals.[86] Three of the cultures showed normal karyotypes, but the two others contained cells with aberrations, 7% in one culture, 11% in the other. Since the EBV-induced aberrations were not clonal, but nonspecific and random, the authors found it easy to distinguish them from the specific and clonal aberrations in the EBV-stimulated cultures from CLL patients.

Prognostic Significance of Chromosome Aberrations
A preliminary report by Robèrt et al. showed a correlation between specific chromosome aberra-



a b

FIGURE 14–13 Translocation between chromosomes 11 and 14, t(11;14) (q13;q32) in CLL. **(a)** Partial karyotype of EBV-stimulated lymphocyte. Q-banding, ×3000. From Gahrton et al.,[41] with permission. **(b)** Diagram of the translocation showing the break points.

tions and the clinical picture of CLL.[99] The time elapsing between diagnosis and the appearance of clinical symptoms that were indications for treatment was significantly shorter in patients with an extra chromosome 12 than in those without this abnormality. It seems therefore that an extra chromosome 12 is associated with a more rapid course of the disease. Nevertheless, the clinical significance of a +12 and other aberrations is still far from clear; additional studies on a greater number of patients and over a longer period of time are necessary before predictions about response to treatment and survival can be made.

T Cell Chronic Lymphocytic Leukemia

The T cell variant of CLL is relatively rare, constituting probably not more than 5%–10% of all cases of CLL. Atypical cases of the disease are frequent and classification of the cases can be problematic. Finan et al. investigated seven patients with T cell CLL; three had typical CLL, whereas the diagnosis for the other four was less clear and the clinical picture was more similar to the CLL "small cell variant" of Sézary syndrome.[27] Chromosomally abnormal clones were observed in the blood of all seven patients. No consistent aberration was found, but chromosome 2 was involved structurally or numerically in four cases and chromosome 14 three times as −14 and two times as translocation, t(11;14), and t(14;18). A +12 was found in one case and an isochromosome 17 was also found in one case, an aberration which has been noticed in different hematologic malignancies. It was also observed that clones with chromosomal abnormalities in some patients gave a variable response to different mitogens. Studies on more patients with T cell CLL are necessary before predictions about karyotypic changes and the clinical course of the disease can be made.

Hairy Cell Leukemia

There are few published studies on karyotypic conditions in patients with hairy cell leukemia (HCL). The most extensive report is based on a group of 26 patients.[45,46] This investigation was performed on peripheral blood, grown without PHA, and on bone marrow and spleen samples, prepared directly or after 24 hours. Most patients exhibited a normal karyotype, which was not unexpected because the mitotic activity of hairy cells is known to be low[12,119] and no B cell mitogens were used for stimulation. Therefore, it seems likely that the dividing cells with normal karyotypes represent the normal cell population of those patients.

In two patients in the series, however, a few cells with recurrent aberrations were detected; an extra chromosome 12 in one patient, an extra chromosome of the C group in another (probably a No. 12), and an abnormal No. 12 in the third patient. An extra No. 12 was also found in a patient of Sandberg.[46] This evidence supports the hypothesis that this chromosome may be the most characteristic abnormality of HCL. This is of interest because +12 is also the most characteristic

abnormality in another B cell malignancy, CLL. The significance of other abnormalities observed in HCL, such as an extra No. 3 and a 6q−, is less clear. Both abnormalities are found frequently in other hematologic diseases, 6q− being one of the rather frequently involved markers in lymphoproliferative malignancies.[82,118] Karyotypic studies on more patients with HCL are necessary and will probably provide more information about characteristic chromosome abnormalities in HCL.

Monoclonal Gammopathies

Cytogenetic studies using conventional nonbanding methods have been performed in more than 230 patients with monoclonal gammopathies.[92,93] Results from banded karyotypes have accumulated slowly because of technical difficulties in obtaining metaphases of acceptable quality. Most publications are case reports and only a few[69,93] deal with more than 8 patients. In a recent review[92] about 50 cases were evaluated. Abnormal clones were observed in 33% in one report[93] and 64% in another.[69]

Most of the abnormal clones (75%) are hyperdiploid, but hypodiploidy (19%) and a few cases of pseudodiploidy have also been found. Trisomy of chromosomes 3, 9, and 11 occurs particularly often. Structural aberrations are clearly nonrandom, although a characteristic monoclonal gammopathy chromosome does not exist. Chromosomes 1, 3, and 14 are most often involved in structural changes. Of special interest are two chromosome 1 abnormalities that result in partial trisomy for the region 1q12–1q3, i.e., the same region that seems of importance for other hematologic diseases. A 14q+ chromosome is the most consistent structural aberration in multiple myeloma, found in 30% of all cases with clonal abnormalities. A 14q+ has also been identified in one line derived from plasma cell leukemia. As donor for the 14q+ segment, chromosomes 1 and 11 could be recognized, but in most cases it is impossible to trace the origin of the extra segment. Since 4 of 11 cases with 14q+ have been diagnosed as plasma cell leukemia, it has been suggested that the 14q+ may be a consistent marker of that disease.

Although the number of cases studied so far does not allow far-reaching conclusions, in one study[93] it was noticed that 5 patients with chromosome 1 abnormalities had a shorter survival than the other 19 cases. No other aberration seems to have clinical significance.

Sister Chromatid Exchanges in Lymphomas

Sister chromatid exchanges (SCE) are considered a sensitive indicator for DNA damage caused by various mutagenic and carcinogenic substances,[64] including drugs given to cancer patients. Analysis of SCE has also been used for the study of chromosome instability syndromes, and it has been shown that patients with Bloom's syndrome have a remarkably increased frequency of SCE in lymphocytes.[18,111,112]

There are only a few published data on the rate of spontaneous SCE in patients with malignant disorders. Kurvink et al.[63] studied SCE in 47 patients with malignant lymphoma; 13 untreated cases are of special interest. Their mean frequency of SCE was 12.7 ± 0.9 per metaphase, which was significantly higher than 6.1 ± 0.3 in the 40 controls. The frequency of SCE increased after treatment of the patients with drugs, such as cyclophosphamide, and was higher in those patients who had received the drugs within a period of 4 weeks prior to analysis (12.9 ± 1.0). In patients who had received the drugs 8 weeks or more prior to analysis, the frequency of SCE was lower than in untreated patients (10.1 ± 0.8).

In a study on cultured lymphocytes of patients with CLL,[79] the SCE rate per metaphase was 11.21 for untreated patients and 10.03 for normal controls. The difference was statistically significant. Thus, these findings are in line with the results on SCE in the lymphoma studies, but Haglund et al.[48] found no significant difference in the frequency of SCE in controls and patients who had been treated for malignant lymphoma in childhood. Unfortunately, comparison of results on SCE studies from different laboratories is difficult because of the use of different methods. More studies are needed for confirmation of the elevated rate of SCE in patients with lymphomas.[108]

Geographic Distribution of Specific Chromosome Abnormalities

Mitelman and Levan have drawn attention to the fact that differences seem to exist between tumor-correlated karyotypic changes in different parts

of the world.[81,82] Data from different countries tend to indicate that different oncogenic agents may underlie the development of tumors in different geographic areas.

Information on CLL and ALL are still limited, due to technical problems, however, results from malignant BL and non-BL are highly interesting.[82] In 23 of 24 cases of endemic BL biopsies and cell lines, a 14q+ marker was found, and in 21 of these cases it was due to a translocation between chromosomes 8 and 14. The number is probably even higher, as chromosome 8 can be difficult to identify in metaphases of poor quality. Our own 14 cases of African BL material showing a t(8;14) are not included in this summary.[131] In contrast, among 9 cases of nonendemic BL, only 4 had t(8;14), and the other 5 had variant translocations.

In malignant lymphomas, the 14q+ marker was detected in only 37 of 79 European cases, whereas it was found in 20 of 24 cases from the United States. Of all translocations involving chromosome 14q and an autosome other than chromosome 8, the t(14;18) is probably the most interesting one with respect to geographic distribution of specific abnormalities. The majority of cases with this translocation have been reported in the United States (30%), whereas only a few cases (2.7%) have been found in Europe.[82]

Although the total number of karyotyped lymphomas is still low, the theory of geographically correlated specific chromosome aberrations in lymphomas is highly suggestive.

Discussion

The most characteristic property of the majority of lymphomas, except BL, is a highly complex karyotype. The aberrations are numerical or structural; they are usually numerous and sometimes impossible to identify. In spite of this often confusing situation, a pattern of nonrandomly distributed changes can be recognized. The most frequently recurring numerical aberrations observed are +7 (in BL), +12 (in CLL), +8 (in ALL), and +1 in various hematologic diseases. The most common structural aberrations are 14q+ (in most lymphomas), 8q− (in BL), 11q− (in malignant lymphomas), 6q− (in various non BL lymphomas), and 22q− (in ALL). It seems reasonable to assume that most of these nonrandomly occurring chromosome changes are of essential importance for the origin and development of the specific tumors in which they are observed since most of them have been found either as the only aberrations in some of the disorders or consistently in several tumors.

Extra Chromosomes

There is no doubt that gains or losses of chromosomes play an important role in tumor development, and it has been suggested that they influence the amount of the gene product in the cell, since they directly alter the number of functioning gene copies.[43] However, recent studies on murine T cell leukemias indicate that the explanation is not quite so simple.

In most experimentally induced or spontaneously occurring murine T cell leukemias, abnormal karyotypes are found, with trisomy 15 being the most common and often only chromosome change.[121-125] Analysis of leukemias with partial trisomy 15 has suggested that trisomy of only a specific segment of chromosome 15 is needed for leukemogenesis.[122] It has also been shown that No. 15 chromosomes from high-tumorigenic mice and No. 15 chromosomes from normal or low-tumorigenic mice are qualitatively different. In experiments too complex to describe in detail here, cells from trisomic T cell leukemia were fused with normal fibroblasts or lymphocytes. Since the trisomic T cell leukemia came from a line with normal chromosome 15 morphology (Tikaut), and the normal fibroblast or lymphocyte from a strain carrying a 14;15 translocation (CBAT6T6), it was possible to distinguish the parental No. 15 chromosomes in the hybrids.

It was found that in high-tumorigenic segregants there was an increased number of tumor-derived No. 15 chromosomes and a decrease in the number of normal parent-derived chromosomes (14;15 translocation). Low-tumorigenic hybrids showed the opposite. It was concluded that chromosome 15 of the high-tumorigenic parent had been changed by mutation or proviral insertion, and that amplification of the changed locus and decrease in the gene dosage of the normal locus promoted tumorgenicity.[116] It seems very reasonable to expect a similar mechanism for trisomies in human malignancies as well, even though we do not have experimental evidence.

Translocations

Translocations of high specificity are the next most common chromosome aberrations in lymphomas and in a number of tumors in general. There is no doubt that the two most discussed translocations in human malignancies are the t(9;22) in patients with CML (and other hematologic diseases)[82] and the BL t(8;14) in patients with African BL.[80] The Ph[1] translocation is not quite understood.

With respect to the BL translocation, it had been suggested that chromosome 14q+ might be the essential chromosome change in the development of the disorder. This hypothesis was grounded on the finding that the abnormality was seen in an overwhelming number of cases, mainly of B cell origin, despite the fact that the extra material on 14q could be traced to different chromosomes. According to a recent review by Mitelman,[80] a 14q+ marker had been identified in 149 of 1871 cases of premalignant and malignant lymphoproliferative diseases, which was in remarkable contrast to only 6 cases with 14q+ in myeloproliferative diseases and solid tumors. (The underrepresentation of a 14q+ marker in T zone lymphomas[44] was unexpected and requires further studies before it can be discussed.) The consistency of the break point in 14q32 was seen as further evidence for the theory that chromosome 14q+ was the crucial chromosome in BL and perhaps B cell malignancy development.

However, the recent detection of variants t(2;8) and t(8;22), has changed the conception of the predominant role of chromosome 14. At least 14 cases of variant translocations have been found and they all have one property in common: both chromosomes 14 are normal and the translocations involve either Nos. 2 and 8 or Nos. 22 and 8. This is strong evidence for the suggestion that in these cases chromosome 8 and not chromosome 14 is the crucial chromosome. The remarkable consistency of certain translocations in correlation with malignancies raises the important questions as to their significance and function.

The BL translocation and its variants have been studied with respect to the localization of the gene locus for immunoglobulin production in man and mouse. It has been shown that human chromosome 14 carries the locus for the production of immunoglobulin heavy chains.[20,21] Furthermore, the human kappa gene locus has been localized to chromosome 2[70,78] and the lambda locus to chromosome 22,[26,78] i.e., the two chromosomes involved as recipients of the distal region of the 8q fragment in the variant BL translocations. The fact that the t(2;8) has the break point on chromosome 2 in the region 2p12, i.e., the region to which the kappa gene has been localized,[70,78] supports the hypothesis of the correlation between gene loci for immunoglobulin production and tumor development.

Here again, the situation in mice may be of help for understanding. In murine plasmacytomas, two characteristic translocations have been found. The most common alteration is a translocation between chromosomes 12 and 15. Chromosome 12 is known to carry the immunoglobulin heavy chain locus in the mouse.[61] Another translocation, found in a minority of cases, is a reciprocal exchange between chromosomes 15 and 6, RcpT(6C;15D3).[89] Tumors carrying this variant translocation produce kappa light chain immunoglobulin. Chromosome 6 carries the kappa locus, although the exact location is unknown.[61] The parallel between the mouse plasmacytoma translocation and the BL translocation is obvious.

Klein proposed a suggestive hypothesis that might contribute to our understanding.[61] According to this theory, a translocation of an oncogene to a highly active locus of immunoglobulin production might bring the oncogene under the influence of an active promotor. As a consequence, an overproduction of some important regulator could be switched on. In the mouse plasmacytomas, the translocation of the distal part of chromosome 15 to the chromosomes carrying the loci for immunoglobulin production rather than the deletion of chromosome 15, would be responsible for the malignant transformation. In BL the translocation of the distal part of chromosome 8 to the immunoglobulin-carrying gen loci on chromosomes 14, 2 or 22 would be the important event leading to tumor development.

Recently the localization of human oncogenes on specific chromosomes has caused great excitement. The first oncogene found, c-myc, was localized to 8q24, the break point in BL and various other lymphomas. The concordance between oncogene localization and chromosome breaks is obvious. In future more results on oncogene localization will probably help to clarify the role of specific chromosome changes in tumorgenesis.

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CHAPTER 15

Generalized Lymphadenopathy in Homosexual Men

Craig E. Metroka, Susanna Cunningham-Rundles, Joseph A. Sonnabend, Richard D. Fernandez, and Janet Mouradian

Since 1981, an outbreak of community-acquired immune dysfunction has been reported in over one thousand homosexual men.[3,18,26,38] These patients came to medical attention either with opportunistic infections such as *Pneumocystis carinii* pneumonia, ulcerative herpes simplex lesions, disseminated *Cryptococcus neoformans, Candida* esophagitis, and disseminated cytomegalovirus (CMV), or with neoplasms such as Kaposi's sarcoma or malignant lymphomas.

More recently, an increased incidence of generalized lymphadenopathy has been recognized in previously healthy male homosexual patients in large cities.[4,26] The patients typically present with lymphadenopathy of at least 3 months' duration involving two or more extrainguinal sites, in the absence of any current illness or drugs known to cause lymphadenopathy. The lymph node histology is characterized by explosive follicular hyperplasia involving both the cortex and medulla with attenuation of the mantle zone.[28] The experience accumulated to date with generalized lymphadenopathy in this patient population is summarized in this chapter. Most of the data are derived from our experience at The New York Hospital. The data suggest that generalized lymphadenopathy is part of a spectrum of a new disorder manifested by acquired immunodeficiency, autoimmune phenomena, opportunistic infections, Kaposi's sarcoma, and, in some cases, malignant lymphomas.

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Patient Population and Presentation

The characteristics of this patient population, based on the first 45 patients studied at The New York Hospital,[28] are as follows: The patients were all men 20–54 years old (mean 32 years); 36 were white, 5 were black, and 4 were hispanic. All the patients described themselves as homosexual (43) or bisexual (2) with multiple anonymous sexual partners (1–4 sexual contacts with different individuals weekly). Approximately 33% of the patients knew other individuals with acquired immunodeficiency, and approximately 20% of the patients were aware of having had sexual contacts within the last year with individuals who subsequently developed Kaposi's sarcoma, opportunistic infections, malignant lymphomas, or generalized lymphadenopathy. None of the patients had a personal or family history of unusual, severe, or recurrent infections prior to the onset of symptoms.

The known exposure to individuals who subsequently developed Kaposi's sarcoma, opportunistic infections, malignant lymphomas, or generalized lymphadenopathy strongly supports the hypothesis that this syndrome is, in part, caused by an infectious agent.[6] However, we cannot rule out the possibility that these diseases reflect common lifestyle factors or another factor not yet determined. Ninety-five percent of the patients had a history of multiple-drug use, the most frequently used substances being marijuana and nitrites.[28] Although 97% of the patients had a history of multiple sexually transmitted diseases, no single venereal disease or treatment was common to these patients. The similarity of this patient population

in age, frequency of sexual contacts, and history of prior sexually transmitted diseases with other groups of patients who have presented with Kaposi's sarcoma[13,25] suggests that we are dealing with an interrelated population of patients who share similar risk factors.

Clinical Illness

A summary of the presenting clinical symptoms based on the first 45 patients in the study is presented in Table 15–1. Approximately 40% of the patients recalled a viral-like illness, infection with either hepatitis A or B viruses, or infection with amebiasis just prior to the onset of lymphadenopathy. Most of the patients had lymphadenopathy for longer than 6 months. The longest duration, in a study in San Francisco, was 20 years (D. Abrams, personal communication). The most frequent complaint at presentation was easy fatigability, which was usually mild. Intermittent fever greater than 38°C was present in approximately 10% of the patients in the absence of an identifiable source of infection. Other complaints included dermatitis unresponsive to topical steroids for the duration of the lymphadenopathy, and dyspnea in the absence of chest x-ray and physical findings.

On initial physical examination, all the patients had lymphadenopathy involving two or more extrainguinal sites. The axillary lymph nodes tended to be the largest, but involvement included the cervical, occipital, submental, submandibular, supraclavicular, epitrochlear, and inguinal lymph nodes. The lymph nodes were usually discrete but were occasionally matted together and tender, and ranged from 0.5 to 5.0 cm in diameter. Splenomegaly was the next most frequent finding and occurred in 30% of the patients. The spleen typically extended from 1 to 6 cm below the left costal margin and was intermittent in some patients.

TABLE 15–1 Symptoms at the Time of Presentation

Symptoms	Patients (%)
Fatigue	75
Sweating	30
Nightsweats	24
Decreased Libido	20
Myalgias	20
Recurrent "viral-like" illnesses	17
Weight loss	10
Fevers	10
Dyspnea	5
Dermatitis	5
Sinusitis	5
Diarrhea	5
Herpes zoster	4
Diffuse alopecia	3
Neuropathic pain	1

Laboratory Evaluation

The most common hematologic abnormality was anemia. This was followed by lymphopenia, leukopenia, and thrombocytopenia. Examination of bone marrow aspirates in patients with hematologic abnormalities showed four patterns:

1. Hyperplastic bone marrow indicating either peripheral destruction or sequestration of mature cellular elements
2. Severe bone marrow hypoplasia
3. Marked left shift in the myeloid series
4. Infiltration with lymphoblasts indicating a lymphoproliferative disorder

A hyperplastic bone marrow was the most common pattern observed.

Additional laboratory abnormalities included elevation of serum globulins in approximately 20% of our patients. Immunoelectrophoresis showed polyclonal elevations of IgG, IgM, and IgA. Patients with dermatologic manifestations were found to have elevated levels of IgE. Mild elevations in serum transaminases were found in approximately 20% of the total patients. All these individuals lacked the hepatitis B surface antigen and IgM antibody to hepatitis A.

This syndrome was also accompanied by a variety of autoantibodies which accounted for some of the clinical symptoms and laboratory abnormalities. Some of the autoantibodies detected to date are listed in Table 15–2.[29,39] Individual patients had one or more antibodies to target tissue. The presence of antibodies or immune complexes did not correlate well with symptoms. However, the most symptomatic patients tended to have levels of immune complexes over 8000 μg/ml. This level tended to fluctuate and may represent a marker for disease activity. The composition of these immune complexes still needs to be determined, but they probably consist of antibodies directed at multiple antigens. Hypocomplementemia was detected in a smaller number of patients (B. Gordon, unpublished observations).

TABLE 15–2 Autoimmune Phenomena

Antibodies to erythrocytes
Antibodies to HLA antigens
Antibodies to lymphocytes
Antibodies to platelets
Antibody to sperm
Antibodies to coagulation factors
Antibody to interferon
Antibody to double-stranded DNA
Antibody to single-stranded DNA
Antibody to ENA
Rheumatoid factor
Lupus-type anticoagulant
Immune complexes
Cryoglobulins

Studies

Infectious Disease Studies

There was no evidence for a single infection common to all the patients, as demonstrated by bacterial, fungal, and viral cultures from blood, urine, semen, and lymph nodes. Of note is that *Mycobacterium avium intracellularae* has been isolated from the bone marrow and *Cryptococcus neoformans* from the lymph nodes of patients in the absence of granuloma formation. The inability to form granulomas probably reflects the underlying T cell defect.

All of the 120 patients had antibody to the viral capsid antigen of Epstein-Barr virus (EBV) (1:40 to 1:5120). Many of the most symptomatic patients tended to have the highest titer to this antigen. However, none of the patients had a positive heterophil antibody, and the high titer to the viral capsid antigen of EBV most likely represents reactivation of EBV. Most of the patients had antibody to CMV (1:8 to 1:>512) and herpes simplex virus (1:8 to 1:32). The sera of several patients were anticomplementary in the assay for complement-fixing antibody to CMV and herpes simplex virus.

Immunologic Evaluation

Of 120 patients evaluated at our institution, only 5 had cutaneous reactivity to intradermal testing with antigens that included purified protein derivative of tuberculosis, mumps, *Candida,* and *Trichophyton* (C.E. Metroka, unpublished observations).

A reversal of the helper-to-suppressor T cell ratio has been documented in patients with Kaposi's sarcoma,[13,40] opportunistic infections,[13,18,26,38] or generalized lymphadenopathy,[40,43] as well as in asymptomatic promiscuous male homosexuals[43] and symptomatic female prostitutes (J.I. Wallace, personal communication). Impaired monocyte function and abnormal lymphocyte composition was described in one study.[2]

Evaluation of the first 45 patients with generalized lymphadenopathy at our institution showed a marked reduction in natural killer cell activity in 67% of the patients.[26] Depressed natural killer cell activity has been reported to occur only in patients with certain primary immunodeficiency diseases or with malignancies.[21] The inability to augment this activity with interferon in *in vitro* studies indicates that natural killer cells and/or precursor natural killer cells are depleted or functionally suppressed.[41] This also suggests that immunotherapy may have to be directed at more than one level of immunoregulation and that interferon may not be adequate to serve this function.

An acid-labile form of an interferon has been identified in patients with Kaposi's sarcoma and generalized lymphadenopathy.[11] The physical properties of this interferon are similar to those of the interferon found in patients with systemic lupus erythematosus and other autoimmune disorders.[31] It has been suggested that interferon may contribute to these disorders.[11] In another study (S. Cunningham-Rundles, unpublished observations), however, patients with Kaposi's sarcoma who did not have detectable levels of interferon obtained either partial or complete remission of their skin lesions with interferon treatment. This suggests that interferon may be useful in patients who lack circulating interferon and that immunotherapy may have to be individualized depending upon the defects observed.

Studies performed on 100 patients at our institution showed a lower proliferative response to various mitogens (pokeweed mitogen, phytohemagglutinin, and concanavalin A) and antigens (*Escherichia coli, Candida, Staphlococcus aureus,* and CMV) than would be expected of an age-matched control group. A negative lymphocyte response to one activator and a strongly positive response to another was found in all patients. It must be emphasized that there is no one immunologic test that is diagnostic for this syndrome; neither is there a close correlation between the lymphocyte activation studies, the extent of inversion of the

helper-to-suppressor T cell ratio, and the degree of clinical impairment (C.E. Metroka and S. Cunningham-Rundles, unpublished observations). However, the greater depression in lymphocyte response to the microbial B lymphocyte activators suggests that these may be a more sensitive indicator for generalized immunosuppression. A similar impaired proliferative response to the B cell activators has been noted in common variable immunodeficiency disease.[9] This, combined with depressed natural killer cell activity appears to identify patients with acquired immunodeficiency.

We have also observed patients who presented with *Pneumocystis carinii* who had essentially normal *in vitro* lymphocyte studies, but subsequently developed marked depressions in one or more of these tests.[27] This suggests that either the initial deficit is limited to an extremely small subset of T cells or B cells, which is not always detected by our assays and which determines susceptibility to new infections or reactivation of latent viral infections. This, however, indicates that once initiated, the immunologic abnormalities as detected by the *in vitro* mitogen studies were progressive in some patients.

More recently, we have observed that sera from patients with *Pneumocystis carinii* had a strong capacity to block the normal T cell response in healthy control donor lymphocytes.[10] All of the patients with *Pneumocystis carinii* also had abnormal lymphocyte responses to the T cell activators in pooled normal human serum–supplemented cultures. Absorption with sheep red blood cells was effective in removing serum factors blocking normal lymphocyte response to phytohemagglutinin and, in some cases, to allogenic cells where allogenic absorption did not prevent serum inhibition of phytohemagglutinin response. Conversely, allogenic absorption removed serum factors blocking normal response in mixed lymphocyte cultures at least as well as, or better than, sheep red blood cell absorption. Increasing the number of absorptions increased the efficacy of allogenic absorption. The positive effect of allogenic absorptions was observed only when the stimulating allogenic cell pool in the mixed lymphocyte cultures was the same pool as that used in absorptions. This blocking factor has also been detected in patients with Kaposi's sarcoma and generalized lymphadenopathy. The results suggest that both anti–T lymphocyte and anti–major histocompatibility complex–linked determinants are present in the sera of patients with acquired immunodeficiency. They also suggest that the immunodeficiency in these patients may occur at more than one level, and that serum blocking factors may be associated with the development of this syndrome.

We have also observed improvement in the response to the specific mitogens and antigens in patients with generalized lymphadenopathy after a monogomous relationship was entered, suggesting that the immunologic deficits may be partially or completely reversible in some patients in the early phase of the syndrome (C.E. Metroka, S. Cunningham-Rundles, unpublished observations). Our results, however, demonstrate that patients with generalized lymphadenopathy have profound loss of both T cell and T-dependent B cell function as studied in lymphoproliferative assays.

As reported for Kaposi's sarcoma, we found a higher frequency of HLA phenotype DR5 (45%) than would be expected to occur in the general population. It will be of interest to determine whether the DR3 antigen offers patients with generalized lymphadenopathy a relative degree of protection against the development of Kaposi's sarcoma, as previously suggested.[30]

Histopathology of Lymph Nodes

Approximately 100 lymph node biopsies were reviewed at our institution. Some of the patients underwent multiple biopsies at other institutions prior to referral for further management. The lymph nodes were characterized by marked and explosive follicular hyperplasia involving both the cortex and medullary zones (Figure 15–1). The follicles tended to become confluent with increasing size of the follicles, along with partial disruption of the mantle zone. Some interfollicular areas showed active expansion with marked vascular proliferation and increased numbers of plasma cells and immunoblasts.

The cellular composition within the center of the follicles varied. In some cases, the follicles were composed of normal reactive constituents. With greater expansion of the follicles, there was an increase in the proportion of large cleaved cells, large noncleaved cells, and immunoblasts (Figure 15–2). Significantly, these reactive changes were present even in lymph nodes that were normal in size. The follicular expansion was limited in several patients, with progressive activation of the

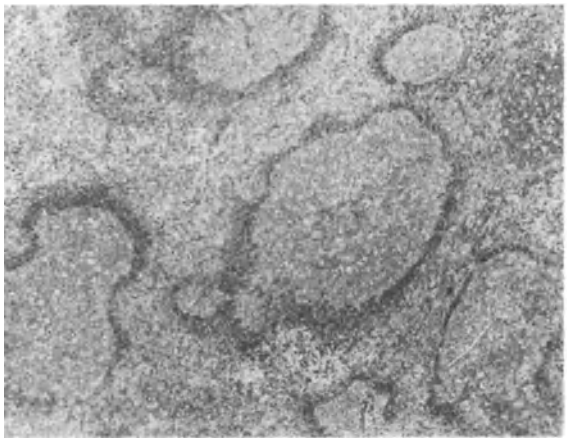

FIGURE 15–1 Low-power photomicrograph of a lymph node showing striking follicular hyperplasia with prominent mantle zone involving both the cortex and medulla. Hematoxylin and eosin stain, ×9.6.

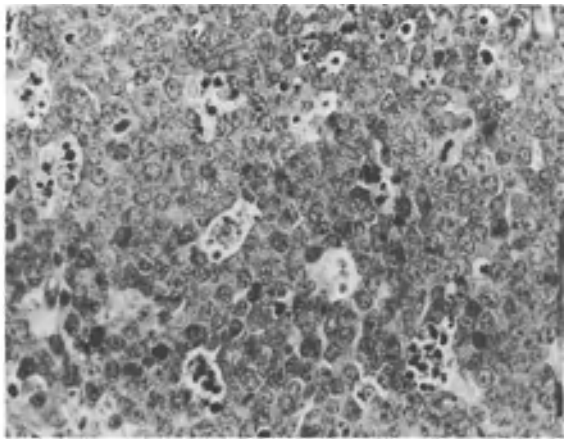

FIGURE 15–3 Burkitt's lymphoma in a lymph node that is completely replaced by uniform neoplastic cells with the characteristic "starry-sky" pattern. Hematoxylin and eosin stain, ×60.

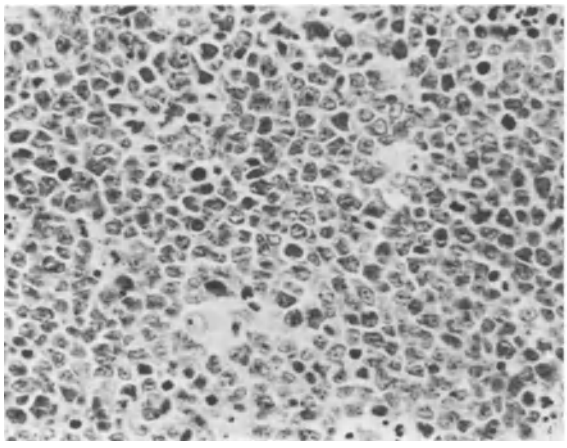

FIGURE 15–2 Photomicrograph of one of many reactive follicle centers, showing the predominance of large cells, both cleaved and noncleaved. Note the scattered macrophages with phagocytic nuclear debris. Hematoxylin and eosin stain, ×60.

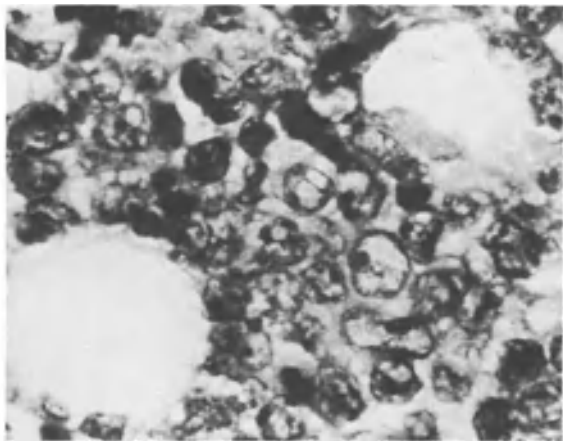

FIGURE 15–4 High-power photomicrograph of a case of immunoblastic sarcoma with large noncleaved vesicular nuclei and prominent central nucleoli. Hematoxylin and eosin stain, ×96.

interfollicular zone resulting in follicular regression with hyalinization of the germinal centers. At the terminal phase of the disease there was neoplastic transformation in several patients, showing Burkitt's lymphoma (Figure 15–3) and immunoblastic sarcoma (Figure 15–4). Kaposi's sarcoma was identified in lymph nodes whose architecture was largely effaced by nodules composed of interweaving fascicles of spindle cells (Figure 15–5). Finally, *Cryptococcus neoformans* was detected infiltrating the lymph nodes (Figure 15–6).

The reactive changes found in the lymph nodes were reminiscent of the findings in graft-versus-host disease,[16,23] X-linked lymphoproliferative disease,[32-434] chronic infectious mononucleosis,[19,33] and the lymphoproliferative disorders in renal transplant recipients.[15,22] Immunofluorescent, serologic, and DNA hybridization studies have suggested that EBV is responsible for these lymphoproliferative disorders,[20,33,35] and it has been suggested that EBV plays a central role in the production of lymphadenopathy in these patients.[28]

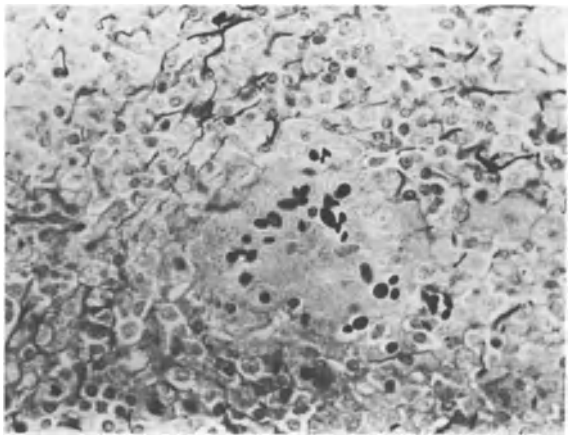

FIGURE 15–5 Photomicrograph of a lymph node largely replaced by metastatic Kaposi's sarcoma characterized by spindle cells growing in various directions, surrounding and lining slitlike vascular spaces. Hematoxylin and Eosin stain, ×24.

Conclusions and Speculations

Generalized, persistent lymphadenopathy has recently been described in male homosexuals.[4,28] The results accumulated to date indicate that generalized lymphadenopathy is associated with significant immunologic abnormalities and may be a precursor to a life-threatening opportunistic infection, malignant lymphomas, and Kaposi's sarcoma. All of the available evidence indicates that the primary event is an acquired immune dysfunction.

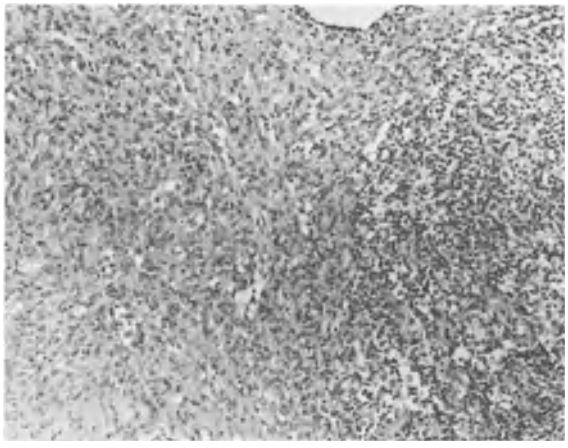

FIGURE 15–6 Photomicrograph of a lymph node illustrating one of many multinucleated giant cells containing numerous round and oval yeast forms of *Cryptococcus neoformans.* gomori-methenamine silver stain, ×60.

A proposed minimal evaluation of patients with generalized lymphadenopathy or suspected acquired immunodeficiency is presented in Table 15–3. Antibody to the viral capsid antigen of EBV should probably be included in this evaluation. However, other infectious causes, such as syphilis, acute infectious mononucleosis, CMV infection, and hepatitis must be ruled out before an extensive work-up is undertaken. As emphasized earlier, this disorder is accompanied by multiple clinical manifestations and has no diagnostic tests. A lymph node biopsy should be performed on any patient with generalized lymphadenopathy of greater than 3 months' duration in the absence of an identifiable cause. This is particularly important in any patient who is symptomatic or if there is a rapid increase in the size of the lymph nodes. Infections such as *Mycobacterium avium intracellularae* and *Cryptococcus neoformans,* as well as malignancies such as Kaposi's sarcoma and malignant lymphomas, have been diagnosed on lymph node biopsy.

Finally, this group of patients would be expected to have a similar frequency as compared to an age-matched control group of other malignant disorders, such as Hodgkin's disease, whose presentation may be indistinguishable from that of acquired immunodeficiency. One patient referred for evaluation was found to have Hodgkin's disease on lymph node biopsy (C.E. Metroka, unpublished observations). On the other hand, *in vitro* lymphocyte studies should probably be limited to medical centers actively involved in patient care or interested in perspectively studying these patients. The indiscriminate use of these tests will only increase the level of anxiety in the general population and will not contribute to our understanding of this disorder.

Recently, a theory was proposed that accounts for the heterogeneous aspects of this syndrome.[28]

TABLE 15–3 Proposed Minimal Evaluation of Patients with Generalized Lymphadenopathy

Intradermal skin testing with recall antigens
Complete blood count, differential, platelet count
Erythrocyte sedimentation rate
Liver function tests
Uric acid
Serum globulins
Serum protein electrophoresis and immunoelectrophoresis in patients with elevated serum globulins
Serologies for EBV
Lymph node biopsy

It was suggested that reactivation of EBV plays a central role in the production of lymphadenopathy and is instrumental in the production of autoantibodies. This was suggested by the histologic features, similarities between this syndrome and those mediated by EBV, and the elevated level of antibody directed against the viral capsid antigen of EBV. Many of the most symptomatic patients had the highest titer to this antigen. Elevated antibody to the viral capsid antigen has been reported in patients with Kaposi's sarcoma[14] as well as in patients with various lymphoproliferative disorders.[24] However, breakdown of host immunity appears to be a prerequisite for EBV-induced lymphoproliferation to occur. Guarding against this are neutralizing antibodies to viral antigens, memory T cells, and natural killer cells.

It is suggested that there are repeated episodes of EBV reactivation in these patients by factors encountered in a promiscuous lifestyle. Initially, the immunosuppression may be reversible. However, as mechanisms for containing the EBV infection become impaired, the number of B cells infected with EBV increases and the repertoire of antibodies also increases. CMV, because of its prevalence in the homosexual population[12] and its profound immunosuppressive effects, must be considered a candidate in the initiation process. It has been observed that when antigens are presented orally to the gut-associated lymphoid tissue, suppressor cells may dissociate widely from gut- to non–gut-associated lymphoid tissue, inducing a specific hyporesponsiveness to that antigen.[1,45] Consequently, the route and the frequency of presentation may be important in causing the initial immunosuppression. The degree of immunosuppression may be further augmented by the presence of immune complexes, nitrites, seminal fluid, and intercurrent infections. All of these have been shown to cause immunosuppression and may produce their effects by different mechanisms. Finally, as the disease evolves, malnutrition and cachexia undoubtedly further immune deficiency.

Any role that semen or nitrites play, however, is probably secondary, since homosexual practices are not new and some of the patients that we have studied denied drug use. The generalized immunosuppression that results could arise from the activity of nonspecific suppressor T-cells, B cell involvement or from the failure of memory T cells to control this proliferation. This picture is reminiscent of many of the features in infectious mononucleosis.[33,36,42] This model takes into account the heterogeneous aspects of the disease, some of which are mediated by autoantibodies. Autoantibodies can form against constituents of blood and result in cryoglobulinemias, rheumatoid factor, immune complexes, anemia, lymphopenia, thrombocytopenia, and bleeding disorders. All of these have been observed in our patients and may be regarded as "epiphenomena" since they are not present in all patients and may occur after variable periods of time.

The immune dysfunction would thus begin as an asymptomatic acquired immune abnormality,[43] and in a smaller subset of affected individuals, eventuate in autoimmune phenomena, systemic symptoms, generalized lymphadenopathy, Kaposi's sarcoma, opportunistic infections, and malignant lymphomas. The clinical manifestations may occur rapidly or after a prolonged latent period. The course would be dependent upon functional disturbances in subsets of T lymphocytes, genetic factors, and exposure to agents that could augment the level of immunosuppression. The types of opportunistic infections may also be determined by organisms endogenous to a particular part of the country. It has been proposed that Kaposi's sarcoma, generalized lymphadenopathy, opportunistic infections, and, in some cases, malignant lymphomas are part of a spectrum of the same disorder. The malignant lymphomas that have been observed may represent an opportunistic form of EBV-induced B cell neoplasia.

While this syndrome has been identified in male homosexuals, there is no reason to assume that it will be limited to this population. Opportunistic infections have been described in female prostitutes (J.I. Wallace, personal communication), intravenous drug abusers,[26,27] hemophiliac patients,[8] and, more recently, the Haitian population.[7] These findings further support an infectious etiology in at least initiating the immune defects. We also cannot rule out the possibility that these diseases have been present but have been unrecognized by the medical community. In support of this is our observation that *Pneumocystis carinii* and other opportunistic infections have been identified in autopsies of intravenous heroine abusers who died 5–10 years prior to the recognition of the present syndrome of acquired immunodeficiency (C.E. Metroka, J. Mouradian, R. Fernandez, J. Sonnabend, unpublished observations).

At present, it is not clear whether the underly-

ing immunodeficiency is reversible. Neither do we know the natural course of this disorder. It is possible that lifestyle factors may be modified. The complications observed to date are listed in Table 15–4. While infections with *Brucella spp., Nocardia steroides, Coccidiodes immitus, Blastomyces dermatitis, Strongyloides stercoralis,* and disseminated adenovirus have not been reported to date, these infections should be anticipated since they occur in patients with defective cellular immunity. Moreover, the higher frequency of severe *Tinea versicolor* and *Molluscum contagiosum* may also reflect the underlying immunodeficiency and these may be regarded as opportunistic infections. However, with the exceptions of Kaposi's sarcoma and lymphoproliferative disorders, most of the opportunistic infections occur shortly after the onset of lymphadenopathy (C. Metroka, and D. Abrams, unpublished observations).

TABLE 15–4 Complications in Patients with Generalized Lymphadenopathy

Infectious complications
Oral thrush
Candidal esophagitis
Herpes zoster
Pneumocystis carinii
Perirectal herpes simplex
Disseminated *Cryptococcus neoformans*
Cryptosporidiosis
Progressive multifocal leukoencephalopathy
Mycobacterium tuberculosis
Mycobacterium avium intracellularae
Toxoplasma gondii encephalitis
Histoplasma capsulatum
Disseminated cytomegalovirus
Legionella spp.
Listeria monocytogenes
Recurrent pneumonococcal sepsis
Staphlococcus impetigo
Hematologic and neoplastic complications
Severe anemia
Severe lymphocytopenia or leukopenia
Severe thrombocytopenia
Lymphoproliferative disorders
Malignant lymphomas
Bleeding disorders
Kaposi's sarcoma
Squamous cell carcinoma of the tongue
Other complications
Renal failure
Progressive blindness secondary to uveitis and/or retinitis (perhaps due to cytomegalovirus or toxoplasmosis)
Progressive dementia (perhaps due to cytomegalovirus encephalitis)
Motor signs (perhaps due to cytomegalovirus encephalitis)
Drug hypersensitivity
Neuropathic pain
Severe dermatitis
Alopecia
Arthralgias
Systemic lupus erythematosus
Leukocytoclastic angiitis

If treatment is to be considered, it should be administered at a time when the immunologic defects are still reversible. Proposed methods of treatment include plasmapheresis, augmentation of natural killer cell activity, augmentation of specific T cell subsets, and the use of antiviral agents. Plasmapheresis has been shown to be of benefit in patients with various autoimmune disorders,[44] and one study demonstrated marked improvement in clinical and immunologic parameters in a patient with steroid-resistant angioimmunoblastic lymphadenopathy.[17] Plasmapheresis would be expected to remove the antigenic stimulators for the hyperimmune state as well as antigen–antibody complexes that can inhibit cell-mediated immune responses. Studies using some of these treatment proposals are presently underway in a number of medical centers.

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CHAPTER 16

Psychological Care of Patients with Cancer

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As physicians have gradually improved the treatment and prognosis of patients with cancer, they have become increasingly interested in their patients' quality of life. Knowledge of the emotional and behavioral responses of patients, once gleaned mainly by an intuitive or commonsense approach, can now be approached more systematically. Use of this knowledge can enhance patients' morale and well-being as they confront a still dreaded disease and can improve physicians' competence and confidence as they treat severely ill individuals.

The presentation in this chapter is not intended to be encyclopedic; it focuses on common situations encountered in the treatment of patients with cancer. The discussion is responsive to questions about patient management that have been posed by colleagues in clinical conferences and individual meetings. The suggestions offered can be incorporated into the mode or style of the oncologist or other practicing physician who is sensitive to the emotional needs of patients.

As Engel has urged, it is time for medicine to change its conceptual approach from the biomedical model to the biopsychosocial model of illness.[24,25] In addition to studying the anatomic and biochemical alterations of the sick, it is advantageous to encompass the psychological and social factors related to the causation, potentiation, and amelioration of their condition. Expressed in other terms, the basic conceptual approach is to consider the patient as a human being and not simply as the host of a disease.

General Approach to the Patient

This approach can be employed from the initial contact with the patient. It can readily be applied with the traditional methods of the physician—obtaining a history, performing a physical examination, and initiating treatment.

In taking a history, physicians can be alert to two tasks simultaneously. On the one hand, they are eliciting the order of development of symptoms. On the other hand, they are attempting to determine how the personality and past experiences of patients will affect their responses to the illness and their capacity to participate in the treatment. In the first meeting with a physician, patients often intersperse much information about themselves as they describe their symptoms. Physicians who pay attention to the personal data can absorb and integrate them into a total view of the patient. Tactful inquiry is sometimes required to supplement the material that is offered spontaneously. Most patients appreciate this kind of interest; it indicates that the doctor cares about their lives, not only about their tumor or abnormal blood count.

Beyond the usual identifying data concerning age, ethnic or racial background, and marital status, one should attempt to learn about the patient's personality, work, family, and interests. In regard to the patient's personality, one need not use technical psychiatric terminology to form some idea of leading traits or characteristics. For example,

does the patient appear to be unusually dependent? Does he or she speak in a distrustful manner? Is this someone who sees himself or herself as the victim of "bad luck" or "hard fate"? Does the patient appear emotionally volatile or stable? Such an initial evaluation, even if only approximate and subject to later correction, will give the physician clues to a suitable approach to the patient.

The composition of the patient's family and the patient's relationships with family members are crucial areas of information. One may need to know, from the very beginning, whether the patient can rely on outside persons, such as the spouse, parents, children, or friends, for emotional, financial, and other types of support. Sometimes data about the patient's relationships with others, particularly parents, will give a meaningful indication of how the patient will react to the physician.

In taking the family history of illness, the physician is usually interested mainly in the genetic or hereditary aspects. In addition, patients often supply a wealth of information about their reactions to the illness and, where applicable, the death of persons close to them. These responses may harbinger their reactions to their own disease, as exemplified by the following case.

A young married man became depressed during his long struggle with a lymphoma, marked by several remissions on chemotherapy followed by recurrences. In the course of interviews, he revealed that his father had died when the patient was 12 years old. Prior to his death, the father had become progressively debilitated, had stopped working, and had withdrawn from his family. The patient was left with a lasting feeling of disappointment and shame about his father. He was now concerned that his own sons of school age would regard him as a weak, inadequate, and deficient father. Further inquiry revealed that the patient had actually conducted himself in a stoic fashion, working whenever he could despite fever, weakness, and other symptoms, and had remained devoted and attentive to his wife and children. Clarification of his fears and pointing out the disparity between his conception of himself as appearing inadequate and his actual performance, helped the patient overcome his depression and persevere during difficult periods of chemotherapy.

The physical examination is such a routine procedure for physicians that they sometimes forget that it is fraught with anxiety for many patients. Some individuals, for example, are secretly worried about the appearance or function of some part of the body. They do not reveal their concern directly during the history taking but allude to it by some seemingly inconsequential remark during the physical examination. Some patients experience feelings of shame and anxiety when the sexual organs and the rectum are examined. The anxiety can be reduced by a considerate preparatory remark before performing the procedure—for example, "I am now going to do an internal examination."

As part of the physical examination, one should include observations about the patient's mood. Patients may express their anxiety verbally or indicate it through physical signs such as tremulousness, diaphoresis, tachycardia, or pallor. Depression may be signified by a characteristic vertical and horizontal furrowing of the brow and either psychomotor retardation or agitation.

An initial assessment of the patient's sensorium, that is, the clarity of his or her consciousness, perception, and thinking, is particularly important in establishing a baseline, which, if properly recorded, can be used for comparison with subsequent observations. The baseline is helpful later in the course of the illness, when one may be concerned about delirium or dementia secondary to fever, drugs, infection, or metastasis to the central nervous system.

It can be rewarding to physicians to test their mettle in observation and description in writing down such data about patients. The oft found phrase in hospital charts—"This is a wd, wn alert male in nad"—is frequently not only imprecise but inaccurate. The patient may be described as "alert" when he is conscious and responds to simple questions even though, on further examination, he may be found to be grossly disoriented, not knowing the name of the hospital or the year. The abbreviation "nad"—no acute distress—is also often misleading. It is sometimes recorded perfunctorily even when the patient has entered the hospital—from his standpoint—because of intolerable pain. Furthermore, it does not take into account the considerable emotional distress, manifested as anxiety, depression, or demoralization, felt by some people on being hospitalized.[81] On the other hand, when physicians are alert to such data, they are not only making a contribution to the description and understanding of the patient's medical condition per se, they are also recognizing the validity of what the patient feels and is experiencing.

As part of the initial diagnostic workup, oncologists often perform or order a large array of tests—blood tests, bone marrow biopsy, lymph node biopsy, x-ray studies, sonograms, and computerized tomographic scans. To patients who are unini-

tiated to these procedures, they are often confusing and frightening. Explanations of the reasons for the procedures and what they actually entail often help to reduce anxiety. If pain or discomfort is involved, patients are thereby prepared for it; or if they are minimal, the patients are reassured. An estimate of the time required in the procedures is also helpful. Explanations are sometimes omitted for the elderly, the confused, and the uneducated on the grounds that they would not understand anyway; but, to the contrary, they are particularly important for such individuals, who are especially prone to misinterpret what they do not understand. The explanations, however, must be geared to their level of comprehension.

In short, certain general principles can be applied to standard medical practices to broaden their scope to include the psychological and social aspects of patient care. The history is enlarged to incorporate pertinent data about the individual's personality, family, work, and level of social functioning. The physical examination includes data concerning the patient's emotional state and cognitive functioning. An important point is that although the mental status examination may be considered a part of the physical examination, a good portion of the observations are actually made while one is taking the history. One may note, for example, that a patient comes close to tears as he reveals his apprehension about not being able to work. Along a different line, one may make observations about a patient's memory as she recounts the events and dates about her own illnesses and those of her relatives.

By taking the added interest in patients' psychological functioning, one is, in effect, eliciting their sense of collaboration in the forthcoming steps of diagnosis and treatment. That collaboration is facilitated by the explanation of each step as it arises. At the same time, the patients' anxiety and potential for feeling manipulated, or being "used for an experiment," are reduced.

Problems at Each Stage of Illness and Suggestions for Management

Initial Diagnosis

The patient's learning that he or she has leukemia, lymphoma, or any other serious illness often constitutes a trauma in the psychological sense. When one thinks of a physical trauma, one conjures up an image of a physical blow, of violence, and of serious injury that the recipient has difficulty in withstanding. With a psychological trauma, the emotional stress is overwhelming, and the impact is more than the individual can endure at the moment. The resulting manifestations are highly varied;[39,83] they often consist of intense anxiety, sometimes to the point of panic, disorganization, or flight from the scene. Other common reactions are incredulity, numbness, anger, depression, and despair.

The physician can help attenuate these distressing reactions through compassion, sensitivity, and careful management.[10,38,85] First of all, one need not impart all of the information abruptly or at one time. A preparatory statement is often helpful in allowing the patient to "gird his loins" emotionally, or to muster his psychological defenses. The type of statement will depend on the style of the physician and the characteristics of the patient. Some examples are as follows: "The test results did not turn out as favorably as we had hoped." "I am sorry to say that some abnormal cells have been found in your bone marrow." "We had hoped that this would be a benign condition, but it has turned out to be more serious."

Such a preparatory statement allows the patient to anticipate an unfavorable diagnosis. What the physician does next, in terms of content and tone, should depend on observations of the patient's reactions. If the patient shows undue anxiety at this point, the physician may offer appropriate reassurance and defer imparting more facts. If the patient, on the other hand, appears to be coping reasonably well, the physician may go on with more specific information.

Dividing the delivery of discomfiting news over a number of visits may help some patients to accommodate to the situation. In others, of course, the tension may be unbearable. One has to individualize one's procedure according to the patient.

In general, one should not impart stressful information, such as "You have leukemia," over the telephone. It is more appropriate to have patients come into the office, where one can observe their reactions and provide suitable explanations and comfort. Often it is helpful to have another member of the family present to share in the reception of the diagnosis and to be available for emotional support.

Conveying hope is of paramount importance.

One should follow up informing the patient of the diagnosis with a message of optimism tempered with realism. Many people fear the worst when they hear that they have cancer, believing they have an untreatable, incurable disease that will lead inexorably to death. It is necessary to emphasize that treatment is available and helpful when this is the case. One need not, however, go into extensive detail initially, since many people are emotionally unready to absorb a large quantity of complex, technical data. What is more relevant is to convey that one does have a treatment plan that will be beneficial. One should allow time to answer patients' questions and rectify any fallacious ideas about cancer and treatment, e.g., that chemotherapy is reserved for terminal cases. Sometimes patients bring out their recollections—often, distorted perceptions—of relatives or friends who have died of cancer. It is beneficial to correct misconceptions and to help patients differentiate between other persons' illnesses and their own.

The fear of abandonment, one of the most universal and deepest of human concerns, may arise at the moment that patients learn of their illness, although they may not express it. If the physician expects to continue with the patient's care throughout the illness, it is reassuring to indicate this intention.

The preceding discussion assumes that the physician intends to inform the patient of the diagnosis of cancer. That assumption is based on the prevailing practice of most physicians in the United States at the present time. It is well to remember, however, that this has not always been the custom in this country and is not the current mode in a number of other countries. A study done in 1961 showed that 88% of physicians surveyed at Michael Reese Hospital, in Chicago, Illinois, usually did not tell their patients that they had cancer.[62] In 1979, a questionnaire presented to physicians at Strong Memorial Hospital, in Rochester, N.Y., revealed an almost complete reversal of those figures.[61] In the latter study, 98% of the physicians surveyed usually did tell their patients that they had cancer. The change in the prevalent practice is due to a more optimistic view of cancer treatment, the need for informed consent, and a general societal trend toward more openness.

There is much to be said for informing patients accurately about the diagnosis. Informed patients are able to participate in the decision whether to undertake treatments that are often quite rigorous and even dangerous. They are prepared, consequently, to cooperate more intelligently with the recommended diagnostic and therapeutic procedures. Physicians, moreover, do not feel that they have to deal with a deception when they have been honest and candid.

On the other hand, one might question the wisdom of adopting a uniform way of proceeding for all people. After all, just two decades ago, physicians of no less competence or integrity usually did not inform their patients; and some physicians in the United States and many who practice abroad still are not candid. Hence, ideas of what is appropriate may be relative to the culture and time in which one finds oneself.

Perhaps it is preferable not to react automatically and apply the commonly accepted method to every patient but rather to consider what is best for each particular individual. For some patients, an ambiguous explanation, e.g., that they have a "tumor," may be indicated to permit their denial to continue even while they embark on a treatment plan. Informing them of the full truth may have to be postponed for some time or even indefinitely.

Treatment Phase

The principle of individualization, discussed previously in regard to informing patients about the diagnosis, applies even more definitely to planning the treatment regimen. This principle poses a challenge to practitioners to utilize not only their extensive knowledge of therapeutics but also their ingenuity in finding the most appropriate regimen for a particular patient. In application, it means going beyond answering the question "What is the optimal treatment for this condition?" to determining "What is the optimal treatment for this condition found *in this patient?*" Experienced clinicians are accustomed to modifying the so-called optimal regimen because of physical complications. For example, one might avoid using doxorubicin in a patient with impaired cardiac function and cisplatin in someone with compromised renal function. Similarly, one may be required to modify the treatment plan if it engenders intolerable fear and aversion on the part of the patient. Otherwise, the patient may refuse treatment or go elsewhere.

Chemotherapy

Nausea and vomiting, loss of hair, weakness, and lassitude are the physical symptoms that most dis-

turb patients. While many of the drugs used in chemotherapy have a potent pharmacologic effect in producing nausea and vomiting, emotional factors often play a role in potentiating those symptoms.[15,29] Any procedure that lessens the patients' anxiety, whether about the illness and treatment themselves or about outside factors, may help decrease the nausea and vomiting. In some instances, frank discussion with the primary physician and the opportunity to ventilate their concerns, will serve to reduce patients' tension. Various drugs, particularly the phenothiazines,[29,30] δ-9-tetrahydrocannabinol,[14,30] and metoclopramide,[32] are frequently of value. For some patients who might ordinarily be treated in the office or clinic, hospitalization may be indicated because of the emotional support provided by a 24-hour medical and nursing staff and the relief from stresses in the home environment.

The loss of hair is one of the most emotionally disturbing experiences found in patients undergoing chemotherapy. The symbolic meaning of the alopecia has import beyond the actual physical phenomenon. To many patients, it signifies a loss of beauty and acceptability to others. To some people, it is a very tangible constant reminder that they are receiving chemotherapy and have cancer. Still, the reason alopecia is so distressing to many individuals is not fully understood and should be the subject of further research. As to practical management, it is advisable from the outset not only to inform patients that they will lose their hair but also to reassure them that the hair usually grows back after the cessation of the chemotherapy. It is helpful to suggest that they obtain a hairpiece before starting treatment and, where feasible, to recommend a skilled wigmaker who has had experience with patients with cancer.

Radiotherapy

The preparatory stage, in which the portals for irradiation are outlined with a dye on the body, is sometimes a period of marked anxiety. Up to this point, patients may not have assimilated totally the fact that they are afflicted with cancer. Then, when the body is marked with the dye, the final denial is abrogated, and anxiety is manifest, occasionally to the point of panic.

The radiation procedure itself is fearsome to many people because of the strangeness of the equipment, the impersonal quality of the surroundings, and the sensory deprivation. These fears can be allayed by explanations ahead of time, having the patient meet with the x-ray personnel and become acquainted with them, and maintaining communication while the patient is undergoing the treatment.

Finally, the completion of a course of radiotherapy precipitates an upsurge of anxiety for some people.[41] This phenomenon is related to the fear of separation from the personnel, on whom the patient has become dependent, and the associated fear of what may happen with the absence of the treatment and the associated "protectors." Similar reactions are seen also in some patients as they come to the end of a course of chemotherapy.

Surgery

Although the technical advances in surgery and anesthesia have vastly improved morbidity and mortality rates, patients still often approach an operation with anxiety. The degree of anxiety and the reasons for it differ markedly from individual to individual.[5] Some people are very frightened of "going under" during anesthesia, the loss of consciousness portending the "endless sleep" of death. Others feel threatened by the loss of control, fearing that they may say or do something bizarre when they do not have full control of their faculties.

Many patients are afraid of pain. Some place the emphasis on whether they will be able to tolerate the pain. Others, however, are more concerned about their behavior—that is, whether they will be able to conduct themselves in a stoic or heroic manner postoperatively as they encounter physicians, nurses, and relatives.

The fear of mutilation is widespread. In operations involving the removal of a breast,[42] the uterus, or the testicle, patients frequently have worries about their sexual attractiveness and apprehension about their being able to maintain their relationships with spouse and close friends.

When cancer is suspected or diagnosed, a particular aura of dread is noted in many persons. The outcome of the surgery may portend literally life or death to such persons. Sometimes patients ask for "the verdict," suggesting that they view the operation in moral or judgmental as well as medical terms. A "bad verdict" may confirm their idea that they are being "punished" for something they did or thought.

Increased Awareness of the Patient's Emotional Plight

Eliciting Patient's Reactions Patients who are reported as "doing well" may be constructing an artificial facade, behind which lies significant anxiety, guilt, anger, or depression. If patients do not spontaneously express their emotional reactions to the illness or the proposed treatment, some broad inquiries on the part of the practitioner are often in order. Such inquiries may be posed as, "What are your fears?" "Have you had some worries that you have not told me about?" and "What problems do you face?"

Some of the fears that patients commonly harbor have already been mentioned. They include fear of pain, fear of loss of control, and fear of dying and of death itself. Other significant fears are related to loss of attractiveness, loss of loved ones, and loss of ability to maintain one's ability to work. Such a listing, however, does not do justice to the uniqueness and diversity of apprehensions, dreads, and fears of the people who are afflicted with cancer. One cannot know, for example, unless one has information from patients, whether they are concerned that cancer is contagious and that they may transmit it to their spouse or children. To some, the dread of impotence, frigidity, or sterility is of overriding importance. To others, the prospect of being punctured by a needle may precipitate panic. The fear of strangers, a residue of early childhood fears, frequently recurs in the hospital situation. To persons who have a large emotional investment in their intellectual functions, the threat of impairment of mental abilities often leads to marked anxiety, which is augmented by the recommendation to have cranial irradiation or an Ommaya shunt.

Recognition of Guilt Feelings The suffering that patients experience with cancer is sometimes significantly increased by feelings of guilt, usually of an irrational nature. Occasionally, patients express their guilt feelings directly, stating that they believe their illness to be the result of actions or of a way of life. For example, a depressed, withdrawn high-school senior with acute lymphatic leukemia attributed his illness to his having "burned the candle at both ends."

More often, patients express themselves less directly. They give clues to their underlying guilt feelings with such expressions as "I don't understand why I have cancer. I did nothing wrong." The statement "I did nothing wrong" indicates that the patient had the idea that he or she had done something wrong but then dismissed, or negated, the idea. Another expression indicating guilt is the frequently heard objection to treatment "I don't deserve this punishment." Here again, the patient has some perception of feeling guilty and deserving punishment but rapidly negates it. Concomitantly, he or she may also reject the treatment, which is seen as punishment. The patient feels that accepting the treatment would be tantamount to an acknowledgement of the guilt.

It should be emphasized that we are speaking of unconscious guilt feelings, which ordinarily should not be investigated or interpreted by one who is not trained to do so. Recognition of these phenomena, however, will increase the physician's understanding of the problem. Reassurance with assuagement of guilt feelings can be helpful. For example, one might say, "I don't believe you did anything wrong, either. The chemotherapy is not intended as a punishment, even though you may perceive it that way. The medications are designed to help you with your leukemia."

Attention to Concomitant Problems The physician may be alert to patients' emotional responses to the physical illness itself but be less aware of the problems that patients are facing elsewhere in their lives. Depending on their situations, patients may feel beseiged by outside problems that exacerbate their reactions to the illness. The following three clinical vignettes illustrate how family problems stirred anxiety in patients and threatened to disrupt their treatment:

A young married woman who had been admitted to the hospital with the diagnosis of acute leukemia became increasingly agitated and suspicious. She appeared to be perplexed at the blue spots on her arms and legs, which were ecchymoses secondary to thrombocytopenia. She pulled out her intravenous line and argued with the nurses about her treatment. On consultation, she revealed overwhelming anxiety not only about herself but also about her three young children, whom she felt she was neglecting by being in the hospital.

Another married woman, who was suffering from a cerebral metastasis from adenocarcinoma of the breast, had never liberated herself from the influence of her original family. Every significant decision in her adult life had resulted in a conflict between her parents and her husband. Now, when decisions had to be made about her treatment, those old conflicts were once again mani-

fested. The parents and husband vied for control of the patient, who was meantime becoming more helpless and anxious because of the rekindling of conflicts and the deterioration of her mental functions secondary to the metastatic brain tumor.

A young single man was admitted to the hospital because of a tumor of the testis, which was found to be a malignant seminoma. After his parents had been divorced during his adolescence, he had lived with his father and grandparents and had almost completely isolated himself from his domineering, critical mother, whom he could not tolerate. In the crisis situation of the surgery and subsequent chemotherapy, his mother presented herself in an intrusive manner that revived the patient's early feelings of inadequacy, rage, and anxiety to an almost unbearable extent. The patient had periods of withdrawal alternating with angry outbursts during which he threatened to leave the hospital.

In all three of these cases, a psychiatrist was called in consultation. He met with both the primary patient and his or her family to clarify the emotional issues of the patient, the family interactions, and their effects on the treatment. In each situation, sufficient calm and stability were secured to enable the therapeutic measures to proceed without significant interruption. The alertness of the primary physician to the emotional and family complications was necessary to identify the problems and initiate psychosocial intervention.

Relapse

When a relapse occurs, it is often more traumatic psychologically than the original occurrence of the illness. With the original episode, most patients mobilize their physical and psychological resources to fight "the enemy"—cancer. As they witness their symptoms subsiding and enjoy a renewed sense of well-being, their morale is lifted. Then, if symptoms recur, they may experience increased anxiety, depression, and even despair.[10,79] Confidence in the doctors and their treatment methods may wane. Trust may give way to distrust, suspicion, irritability, and outright anger. Some individuals leave their physicians, reject the usually accepted modalities of treatment, and seek relief from laetrile or other unproven methods, often with dire results.

The guidelines for dealing with the emotional consequences of relapse have to be broad and flexible. The situation calls on the psychological resources of physicians to remain calm, patient, and compassionate. In general, an attitude of realism coupled with optimism is recommended. Physicians should first allow patients to express their ideas and feelings about the recurrence of their disease and then gauge their approach accordingly. Some patients may require mainly support and generalized reassurance that help can and will be provided. Others may need the outline of a definite plan or the choice of plans available to meet the new contingencies. Some patients and their families place the emphasis on doing all that is possible, "leaving no stone unturned." Then, consultation with another oncologist may bolster confidence in the primary physician and the medical system. Others may prefer that a minimum be done, ostensibly because the patient "has suffered enough." That attitude may be indicative of underlying conflict and possibly depression, which may require psychiatric consultation.

It is important to bear in mind that, even in the face of recurrence and the threat of death, many patients do reach a state of equanimity. Through various defense mechanisms and coping mechanisms, they go through a process of "mitigation and accommodation";[85] that is, they adapt to a state of chronic illness, disability, and uncertainty about the future.

Terminal Phase

When a patient is thought to be in the terminal phase, is often a matter of definition. At one extreme, some people regard themselves as "dying" from the moment that they learn of the diagnosis of leukemia or lymphoma. At the other extreme, there are individuals who, despite an unmitigated decline in their physical condition, maintain the hope that their health will be restored. The labeling of someone as "terminal" is sometimes made by the physician rather than the patient. Such a designation should be made with caution, since it may prompt caregivers to withdraw from the patient, unless they have been specially trained to cope with their reactions to the dying patient.

Physicians who regard themselves principally as curers of illness may find themselves feeling defeated and helpless as a patient approaches death. They may then declare to the patient, "There is nothing more that I can do for you." That response does not take into account the important sustaining functions that the physician can provide until the moment the patient expires. Relief of pain and other distressing symptoms reduces the patient's suffering. Beyond the relief of symp-

toms, the maintenance of the patient's emotional bond with the physician preserves the feeling of being cared for and protected. Even patients who may no longer have hope of survival should not be deprived of the hope of safety and security for the remainder of their lives.[85]

The field of death and dying has been the subject of an outpouring of books and articles as well as the province of a special journal, *Omega.* Kübler-Ross was prominent in bringing this topic, previously shunned, into the open for discussion and increased understanding.[49] She divided the psychological reactions to dying into five stages: denial, anger, bargaining, depression, and acceptance. The delineation of those stages is useful insofar as it helps to identify the patient's predominant mental set. Many patients, however, experience a combination of emotional reactions at a given time, and most do not go through them in a predictable order. Some never reach the stage of acceptance.

There is, furthermore, a pitfall in this type of categorization in that it may lead one to overlook the complexities and finer points of the patient's emotional state. For example, if the patient is depressed, what are the components of the depression? What is the meaning of debility to this individual? Is there a disruption in communication between the patient and the rest of his or her family? How does the patient regard the remainder of his or her life? What is this person's conception of death? What are his or her specific fears?

In the last few weeks or days of life, some patients sink into a "terminal delirium," in which their perceptions are clouded or distorted. Some physicians regard the terminal delirium as a "blessing," since these patients are then supposedly unaware of what is going on around them and of the fact that they are dying. With this attitude, the physicians may not seek to lighten the delirium by correcting metabolic disturbances or may even increase it by prescribing heavy doses of narcotics or sedatives.

Management again has to be individualized. Perhaps for some individuals it is best to go to the grave in an unknowing state. However, unless patients are severely obtunded or comatose, they are partially aware of the events surrounding them. For example, a delirious middle-aged man dying from cancer of the lung, asked repeatedly, "When are they going to take away the body?" Although quite disoriented and psychotic, he was still painfully aware that he was dying. Furthermore, with the sensorium clouded, patients may misinterpret the actions of those around them or suffer from frightening nightmares or hallucinations. In such instances, a reduction of narcotic or sedative drugs may actually make the patients more comfortable.

In this connection, it is worth noting that some patients who are aware that they are dying have a strong wish to say goodbye to their loved ones.[58] If physicians deal realistically with patients and do not sedate them unnecessarily, the dying may indeed bid farewell to their family and close friends. Parting in this manner may permit patients to die in a peaceful, accepting manner and may also diminish the later suffering of relatives and friends. Physicians cannot stage such a farewell, but they can sometimes help provide the conditions that make it possible.

The "Cured" Patients

A survey of the literature reveals that relatively little has been written about the psychological status of patients who have been "cured" or attained long-term remission from cancer. Nonetheless, some general statements can be made about the susceptibility of "cured" patients to psychological dysfunction.[76] The most prevalent symptom is anxiety about the recurrence of illness. Most patients deal with this anxiety by denial—"It can't happen to me again." The anxiety, however, may break through in overconcern about lumps or minor symptoms in the patients themselves, their children, or other relatives. Sometimes the reaction to illness is manifested in chronic depression, withdrawal, and a lowered level of aspiration.

As a result of severe disease, there is a change in the patient's *self-image,* defined as "the image which an individual has of himself at a particular time in a specific situation."[59] Along with a change in self-image, there is often a fall in self-esteem, the regard one has for oneself as a person. One may then attribute to other persons certain feelings of dislike or disrespect that one feels toward oneself. (See the clinical example on page 307.) This chain of events frequently leads to disturbances in marital and other relationships. Often these difficulties are denied, rationalized, or minimized by patients; and physicians may be unaware of them unless they specifically inquire.

The foregoing problems are vividly exemplified by recent studies of patients who have been suc-

cessfully treated for Hodgkin's disease by chemotherapy.[16-19,82]

> One such patient, a 32-year-old woman, reported to the investigators, who had inquired as to the state of her libido, or sexual drive, "I told the doctor two years ago that I had *no* sexual feeling and that I was worried and could he explain it or do anything about it, and he said, 'Well! You're alive aren't you?'."[21]

The investigators, Chapman, Sutcliffe, and Malpas, began to study systematically the effects of chemotherapy on patients with Hodgkin's disease. They found that ovarian function had failed or was failing in 83% of the women. Concomitantly, these women underwent heightened symptoms of the menopause: hot flushes, irritability, insomnia, dryness of the vagina, and painful intercourse. They had a "poor self-image" and were depressed. They reported either complete absence or marked diminution of sexual desire. In the younger women especially, these symptoms and the lack of sexual interest led to marital discord of major proportions. In 30% of the couples studied, the stresses led to separation or divorce.

The patients were usually unaware of the connection between their previous chemotherapy (mechlorethamine hydrochloride, vinblastine sulfate, procarbazine hydrochloride, and prednisolone) and their lack of sexual desire and other symptoms. Their former physicians had either not inquired about or ignored the patients' complaints. Ultimately, when the investigators realized the nature of the phenomena, they were able to take corrective measures—the use of hormones and counseling—which often produced prompt and gratifying relief.

The male patients whom they studied also manifested decreased libido, infertility, and irritability. In addition, the men showed an increased propensity to physical violence.[19]

Specific psychological problems are found also in other groups of patients who, although cured of cancer, are left with significant physical defects. Important examples are patients who have undergone mastectomy,[42,87] amputation of a limb,[63] or abdominoperineal resection with provision of a colostomy.[22,23]

The plight of long-term survivors of cancer occurring in childhood has been extensively investigated by Koocher and O'Malley, who have reported their findings in a book aptly entitled *The Damocles Syndrome.*[47] The survivors' major problems center around uncertainty.[46] They are faced with uncertainty, and concomitant fear or anxiety, about many issues: fear of death, fear of recurrence of the original cancer or appearance of a second tumor, and fear of other long-term effects of treatment. They have worries about being sterile and questions as to whether they should marry and, if possible, have children. They are apprehensive that their offspring may have cancer or congenital defects. They are concerned about not being able to obtain jobs and insurance because of discrimination toward people with a history of cancer.

Their worries about the occurrence of second tumors have some basis in fact. Of 142 patients, 10 developed second primary malignancies and 7 developed benign tumors.[53] Concern about offspring is a more complicated issue. The question of heredity has to be explored on an individual basis, taking into account the particular type of cancer and the family history. As to the effect of the treatment of patients on their future children, one study, although small, is reassuring. It shows no increase in congenital defects in the progeny of individuals who were treated for cancer during their own childhood.[52]

Many of the patients who are now adults have married, had children, gained fruitful employment, and reached a level of emotional maturity. Others, however, present psychological symptoms and problems in adjustment. In the series of Koocher and O'Malley,[47] 53% of the survivors are considered well adjusted and have no evidence of symptoms, 25.6% have mild symptoms but are functioning adequately, and the remaining 21.4% have moderate to severe symptoms. Unfortunately, no control group of physically healthy people could be provided. Comparison, however, with a small number of survivors of chronic but not life-threatening diseases showed that the patients who had had cancer manifested a larger incidence of psychological symptoms and problems in adjustment than the patients with the other diseases. Within the cancer group, those who had had acute lymphocytic leukemia or Hodgkin's disease in childhood showed the highest probability of later developing problems in adjustment.

In brief, these researches indicate that patients who have survived cancer have an increased propensity for psychological symptoms and problems in their daily lives. The knowledgeable clinician will evaluate psychosocial adjustment during follow-up visits and provide therapeutic intervention where needed.

Specific Psychiatric Syndromes

Patients with cancer are subject to all of the large number of psychiatric disorders, but the conditions most commonly encountered are the depressive disorders, the anxiety disorders, and the organic brain syndromes. The recognition, diagnosis, and appropriate treatment of these entities contribute a vital element to the comprehensive care of patients.

Depressive Disorders

Diagnosis

Many patients experience reactions of depression or grief as they are confronted with life-threatening illnesses and arduous treatment regimens. Gradations in severity are extensive, ranging from mild, transient episodes of feeling "down" or "blue" to major depressive episodes. While there are no absolute distinctions between "normal" and "abnormal" reactions, certain criteria do help to differentiate the less serious from the more pathological conditions. In grief about a loss and in relatively mild depression, patients do not suffer an inappropriate lowering of self-esteem; in a serious depression, they may regard themselves as worthless and unworthy of the respect or love of others.[84] In grief and mild depression, feelings of guilt are absent or minimal; in severe depression, guilt feelings are often prominent. Severely depressed people with a physical illness may blame themselves irrationally for having caused the illness. In some instances, they may attribute that blame to others, angrily accusing them in a paranoid manner.

In the full-blown major depressive disorder, patients suffer from a constellation of symptoms, both somatic and psychological.[31,44] The classic somatic symptoms are anorexia, weight loss, constipation, decrease in sexual activity, and insomnia with early-morning awakening. The psychological symptoms are loss of interest and pleasure in work, recreation, and other usual activities; loss of self-esteem with self-depreciatory tendencies; irrational feelings of guilt; feelings of helplessness and hopelessness; and suicidal ideas, wishes, or actions. Many depressed patients exhibit psychomotor retardation or agitation. Some of the most severely depressed suffer from somatic delusions and delusions of guilt, poverty, and persecution.

In patients with cancer, the diagnosis of depression is complicated by the overlapping of the somatic symptoms of classic depression with the symptoms produced by the cancer itself or the treatment modality.[66] Then one must rely more heavily on the nonsomatic, or psychological, symptoms.

Depression is a symptom occuring in a number of different syndromes. To be understood properly, therefore, it must be subject to a differential diagnosis. That process involves a clinical assessment as to whether the patient's condition falls into the category of a major depressive episode, a lesser depressive syndrome, a grief reaction, or organic syndrome. In addition, the process of differential diagnosis requires seeking out the etiologic factors, both physical and psychological.[31,64]

A number of physical conditions are associated with depression. Sometimes structural changes in the central nervous system, such as those due to primary or metastatic brain tumor or carcinomatous meningitis, are initially manifested clinically by depression. Metabolic and electrolyte disturbances are also connected with depression. Renal failure, hepatic failure, hyperthyroidism, hypothyroidism, Cushing's syndrome, and hypoadrenocorticism are other etiologic conditions. Overuse of narcotics, barbiturates, benzodiazepines, and other drugs may lead to or potentiate depressive tendencies. Notably, the use of steroids, reserpine, and methyldopa is frequently associated with depression.

Sometimes what is mistakenly taken for a functional or reactive depression is actually an organic brain syndrome. Patients who are "confused," disoriented, and having difficulty in ordering their thoughts and memories may indeed appear depressed or actually suffer from depression, but the most significant causal factor or factors are the metabolic or structural derangements. Hence, they must be considered in the differential diagnosis.

Alongside the search for physical causes, one should investigate the psychological factors. One should avoid the dichotomy of ascribing the depression exclusively to either organic or psychological elements. Most often, a multiplicity of factors has to be considered and integrated.

The psychological factors involved in depression may be subsumed under two main categories. The first category includes experiences of loss, threatened loss, and phantasied loss. To the patient with cancer, such losses include the loss of health in a general sense, the loss of particular functions, and, most significantly, the loss of life itself. En-

tailed in those losses are the threatened separations from loved ones, death being the ultimate separation. For someone in the early stages of disease, the losses may involve one's job, one's accustomed position in the family as breadwinner or homemaker, and one's financial security.

The other main category of factors involves the threat to the individual's sense of himself or herself as a person—that is, his or her self-evaluation or self-esteem. The concept of the self is intimately connected with the concept of the body. Hence, if the individual's body is weakening and its functions are failing or threatening to fail, he or she may feel less of a person and suffer from feelings of shame or worthlessness.

In examining why a person is depressed, one should avoid superficial generalizations, such as "It's understandable that the patient is depressed, considering his condition" or "After all she's been through, she's entitled to be depressed."[84] Such generalizations obscure many issues. Not everyone with cancer, even with a devastating course, becomes depressed.[51,66,79] One has to look beyond the severity of the physical manifestations of the cancer to understand the reasons that an individual has become depressed; that is, feelings of guilt and shame, fears of sustaining losses, and many other intrapsychic, somatic, and environmental forces are often at work.

The comment that a particular patient is "entitled to be depressed" may imply a judgmental standard that some other patients, such as those without grossly apparent physical suffering, are not entitled to be depressed. However, one should look as objectively at depression as any other ailment and refrain from value judgments.

Furthermore, in attempting to appraise whether a patient is "entitled" or "has a right" to be depressed, physicians may be attributing to the patient ideas and feelings that originate in themselves. For example, they might think, "If I were in his place, I certainly would be depressed." The pitfall in that attitude, seemingly empathic, is that it may impede physicians from pursuing with the patient the specific factors responsible for that particular person's depression.

Treatment

As indicated by the previous discussion, the first step is to determine the causes of the depression. Any physical abnormalities that are interfering with cerebral function and contributing to depression should be corrected when possible. Furthermore, it is advantageous that the physician have empathy with the patient and be attentive to the various personal, family, and social factors that are influencing his or her mood. Often if the patient can express openly to the physician his or her concerns, that in itself will have a salubrious effect. Clarification in simple terms may help the patient cope better with the situation. A realistically hopeful attitude on the part of the physician and other caregivers is also beneficial.

When correctable physical factors have been removed and simple psychological measures have been provided yet the patient remains depressed, tricyclic antidepressant drugs (imipramine, amitriptyline, etc.) often provide symptomatic relief. Since their pharmacology is well reviewed in many books,[45,75] only a few significant aspects are emphasized here. Patients with severe cardiac disease, especially with conduction defects, pose a special risk with these drugs.[43] In elderly patients, the usual dosage should be reduced to one-half or one-third. Hypotensive episodes, secondary to orthostatic mechanisms, may result in falls with serious injuries and cardiovascular complications.[70]

Patients with severe depression, especially those with suicidal preoccupation and psychotic ideation, should be referred to a psychiatrist.

Anxiety Disorders

Diagnosis

The state of anxiety is characterized by a subjective feeling of apprehension, varying in intensity from mild discomfort to overwhelming dread or panic. In some patients, the syndrome is confined to feelings of psychological distress but, more frequently, those feelings are accompanied by physical symptoms of a wide variety. The most common symptoms are palpitations, difficulty in breathing (sometimes associated with hyperventilation), excessive sweating, tremor, dryness of the mouth, and dizziness. Almost every system of the body can be affected because of the discharge of the sympathetic and parasympathetic divisions of the autonomic nervous system. Some patients are aware only of the physical manifestations and do not connect them with the anxiety state, although they may be anxious secondarily about the physical symptoms (e.g., be frightened that palpitations presage a heart attack). The anxiety may be reflected in a pervasive state of uneasiness and worrisomeness or may occur in discrete attacks (panic disorder). Sometimes they are confined to specific situations,

such as closed-in spaces or heights (phobic disorder).

As with the depressive syndromes, the differential diagnosis is of importance. Physical illness, such as hyperthyroidism and pheochromocytoma, may produce symptoms similar to those seen in patients suffering from psychogenic anxiety attacks.

Anxiety states can mask or be confused with other psychiatric conditions. Some individuals who complain of severe anxiety and exhibit agitation (e.g., pacing the floor, picking at clothes, and wringing their hands) are suffering from a major depression. They may not complain of feeling depressed and may even deny it upon inquiry, but on further investigation they reveal the history of a serious depressive episode. Some patients with severe anxiety are undergoing a major disorganization of personality with incipient psychosis, such as schizophrenia. Other patients with severe anxiety are exhibiting a paradoxical reaction to barbiturates or other drugs, or withdrawal from sedatives, tranquilizers or alcohol. Sometimes anxiety is one of the first symptoms of delirium.

The underlying psychological factors must also be sought out. In patients with cancer, the occurrence of anxiety symptoms is frequently related to a stressful event connected with the illness, such as hearing the initial diagnosis or learning that they must undergo an operation. The time of recurrence of illness is particularly stressful for many individuals and frequently leads to a resurgence of anxiety.[79]

It should be remembered, however, that most persons with anxiety disorders do not suffer from cancer or other physical illness. Psychological study reveals that they often have unconscious fears, such as losing loved ones or losing the approval of loved ones. The fear of physical harm or mutilation also plays a large part in anxiety disorders and related conditions. These fears of loss and bodily injury, often stemming from childhood events or phantasies, are reactivated when the individual is confronted with real dangers, such as those presented by cancer. In short, although they have a basis in present reality, they also have a source in childhood conflicts.

Treatment

For practicing oncologists, the current, realistic factors should be the focus of attention. By education, reassurance, and continued support, they may help to reduce patients' feelings of apprehension and dread. Often misconceptions and misunderstandings about the nature of their disease increase the patients' anxiety, which can be alleviated by appropriate explanation. To be helpful, reassurance should not be supplied too readily or glibly. First, one should attempt to ferret out just what the specific fears of the patient are.

A number of medications are of value in dealing with states of anxiety and insomnia. The principal ones are the so-called minor tranquilizers, especially the benzodiazepines (e.g., chlordiazepoxide, diazepam, and flurazepam). Since the blood level of the longer acting compounds tends to build up in time, some patients taking these drugs develop a delirium. Hence, it is often preferable to prescribe a shorter acting compound, such as lorazepam or oxazepam.

In patients with more severe states of anxiety, where disorganization or psychosis is threatened, one may use a major tranquilizer, either haloperidol or a phenothiazine (e.g., chlorpromazine or thioridazine). In such instances, psychiatric consultation is advisable.

Organic Brain Syndromes

Recent study of the organic brain syndromes has resulted in clarification of the underlying concepts and major changes in classification.[56] A previous classification[2] divided these nosologic entities into acute and chronic brain syndromes on the basis of rapidity of onset, duration, and prognosis, the acute syndromes being considered reversible and the chronic syndromes irreversible. The current classification[4] does away with the distinction between acute and chronic organic brain syndromes, recognizing that prognostic criteria should not be combined with diagnostic criteria and that, furthermore, some of the chronic conditions (for example, those due to neoplasm or neurosyphilis) are at least partially reversible. Another previous distinction in classification—between psychotic and nonpsychotic organic states[3]—has also been eliminated in the new classification[4] as impracticable. In addition, the concept of one basic "organic mental syndrome" has yielded to a more detailed, precise differentiation.

Nine distinct, although sometimes overlapping, types of organic brain syndrome have been delineated.[4] They are delirium, dementia, amnestic syndrome, organic hallucinosis, organic delusional syndrome, organic affective syndrome, organic personality syndrome, intoxication, and with-

drawal. In previous conceptualizations, cognitive impairment was regarded as the sine qua non of the organic brain syndrome. Disturbances in intellectual function are still considered the hallmark of some of these syndromes, especially delirium and dementia; but the new classification recognizes that impairments in cerebral function may produce disturbances in the areas of mood, perception, ideation, and personality without aberrations in cognitive functions.

Of the nine organic brain syndromes, the two that are most commonly encountered in medical practice are delirium and dementia. Both syndromes are characterized by global alteration in the various cognitive, or intellectual, functions[56] but are also often associated with disturbances in mood.

Diagnosis of Delirium

From the clinical standpoint, the essential feature of delirium is clouding of consciousness, characterized by impairment of thinking, perceiving, and remembering.[55] The patient has difficulty in attending to the environment and correctly perceiving objects and events. The more frank distortions are manifested by hallucinations, most commonly in the visual sphere, and delusions. Signs of disorientation to time, place, situation, and person appear in approximately that order, depending on the severity of the condition. Disturbances in the sleep–wakefulness cycle are frequent, ranging from hypervigilance and insomnia to drowziness, stupor, and eventually coma. Associated disturbances in mood and mentation are common, the most frequent being irritability, anxiety, depression, and paranoid ideation.

One of the most fundamental features of delirium is the fluctuating level of awareness.[26,55] This variability is evident not only in the diurnal cycle, with typical exacerbation at night, but also from hour to hour and even from moment to moment, as exemplified by a patient's suddenly losing the train of thought. The fluctuation in level of awareness can be elicited by a simple bedside test—serial sevens. The examiner asks the patient to subtract 7 sequentially from 100 (that is, 100, 93, 86, 79 . . . .). In mild or moderate delirium, the patient alternates periods of easy, correct calculation with periods of making errors despite heightened effort. The electroencephalogram is a valuable tool in the differentiation of delirium from other conditions.[26,55,67]

Delirium is caused by a vast number of factors that alter cerebral metabolism and neuronal functioning. Failure of any of the major organ systems—renal, hepatic, cardiac, or respiratory—results in alterations in brain function. Other systemic conditions are also frequent causes of delirium: anemia, infection, fever, dehydration, acidosis, alkalosis, and deviations in osmolarity. Endocrine and metabolic disturbances can also produce profound effects. Abnormalities in electrolyte levels are often involved in delirium. Hypercalcemia is a frequent cause in patients with certain types of cancer.[86]

Any drug can lead to delirium. The medications most commonly implicated are the opiates, the barbiturates, and the benzodiazepines. In oncologic practice, the steroid compounds, such as prednisone and dexamethasone, are frequently utilized and have a large incidence of mental complications. When the daily dose is 40 mg or more of prednisone (or the equivalent of other steroid compounds), the incidence of organic brain syndromes is notably higher than when the dose is below that level.[11,36] The steroids produce a variety of syndromes—not only the cognitive changes characteristic of delirium but also depression, maniclike episodes, and paranoid states.[36,54]

Other drugs used in the treatment of neoplastic disease are difficult to evaluate in terms of their effects on mental function.[65] Often several chemotherapeutic agents are used in combination, posing problems in singling out those producing delirium. Moreover, alterations in mental state resulting from the disease process and complications, such as infection and anemia, are difficult to differentiate from the effects of drugs. There is, however, convincing evidence of the profound influence of L-asparaginase[40] and methotrexate,[77] the latter administered intravenously or intrathecally, especially when combined with radiotherapy.

Diagnosis of Dementia

In dementia, the key feature is the progressive impairment of memory and other cognitive functions.[56] The onset is usually more gradual than in delirium, and the clinical picture generally does not show the marked fluctuations characteristic of delirium. Besides deterioration in memory, other significant features include disturbances in orientation, judgment, and the ability to carry on the usual tasks of everyday life. In some instances, depression, manic phenomena, paranoid ideation,

and the loss of social inhibition or control are noted.

The most common causes of dementia in patients of the older age group are degenerative changes of the Alzheimer type and multiple cerebral infarcts secondary to vascular disease. Particularly significant to oncologists, however, are some other causes of structural brain damage: primary brain tumors, metastatic brain tumors, leukemic and lymphomatous lesions of the brain, and carcinomatous meningitis. Those conditions often are first manifested by mental symptoms, prior to the appearance of other neurological signs or symptoms.

The syndrome of pseudodementia sometimes presents a problem in differential diagnosis. In this condition, patients complain of loss of memory and give the initial impression of being demented. With more careful history taking and examination, however, they are found to be suffering from a severe depression with concomitant difficulty in concentration. Treatment of the depression usually results in clearing of the cognitive disturbances.

Treatment of Delirium and Dementia

The most important general principle of management of the organic brain disorders is to discover the underlying etiologic agent or agents. Although a distinction is made between dementia and delirium, the two syndromes often occur together. It is not unusual for a patient with dementia to have a superimposed delirium, secondary to physical illness or drugs. In delirium, furthermore, several interacting causative factors, rather than a single agent, are commonly involved.

In the treatment of both delirium and dementia, the primary emphasis is on removing or alleviating the causative structural or functional conditions whenever possible. Abnormalities in electrolyte balance and metabolism should be corrected. A thorough review of the patient's medications is advisable. When feasible, drugs should be discontinued or the dosage reduced.

In addition, supportive measures are indicated. The bewildered, anxious patient often benefits from the care of a constant companion who can provide safety and reassurance and help the patient correct distortions of reality. Keeping the lights on in the patient's room at night helps to reduce the possibility of misperceiving shadows as ominous objects. Sedatives or tranquilizers are generally not necessary or advisable. If, however, anxiety or agitation cannot be controlled by supportive measures, haloperidol or a phenothiazine may be used as a temporary expedient.

Special Treatment Issues

Transference

Through experience with innumerable patients, the physician may be struck with this phenomenon: even though his or her own behavior is relatively uniform, patients react to him or her in extraordinarily diverse manners. Some patients, for example, are immediately trusting and accepting, whereas others are suspicious and questioning. Some are dependent and willing to leave all major decisions to the doctor, while others militantly strive for independence and insist on making the important choices. Some are warm and engaging, but others remain cool and aloof.

In part, these types of behavior represent the patients' character or personality—enduring patterns of thinking, feeling, acting, and forming relationships. The patients may enter into a relationship with their physician in much the same manner as they approach most of the other persons in their life.

In addition, these characteristic reactions are often carry-overs from childhood; that is, the patterns of feeling, thinking, imagining, and acting that the person originally experienced in childhood, especially toward parents, are displaced to various figures in adult life. This phenomenon, referred to as *transference,* was originally elucidated by Freud in his psychoanalysis of neurotic patients.[28] The analysis of transference attitudes, feelings, phantasies, and behavior remains the cornerstone of psychoanalysis and analytically oriented psychotherapy. Its significance, however, extends far beyond the psychiatrist's office. All people, normal as well as neurotic, exhibit transference reactions. Furthermore, patients show these reactions to all physicians, not only to psychiatrists.[13] (See the clinical example in the section on Transference Phenomena, page 323.) As indicated, the type of reaction is highly varied. It is a misconception to believe that transference refers only to sexual feelings. Many other emotions, such as anger, resentment, jealousy, admiration, and denigration, may also stem from early experiences with parents or phantasies about them.

For example, consider a patient who is frustrated and angry. He may, of course, have seemingly valid reasons for his feelings. The treatment may not be producing the desired results, or the physician may have been inadvertently impatient and brusque. However, the realistic reasons may not appear sufficient to explain the magnitude of the patient's reactions. Moreover, sometimes one is hard put to find any plausible explanation for a patient's hostile reaction when the physician has been attentive and the results of treatment have been favorable. One may then find the basic reasons not so much in the current situation as in carry-overs from the past.

There are two main corollaries to these ideas about transference: The first is that physicians can understand not to take their patients' reactions personally. They can recognize that they may be the recipients of attitudes and behaviors derived from the patients' past. The second corollary is that, feeling more comfortable about patients' reactions to them, physicians can learn to respond to patients more objectively and effectively.

In addition to those two corollaries, there are two caveats concerning transference. The first is that, just because physicians can recognize certain patterns of childlike behavior in patients and identify their sources, they are not exempted from reflecting upon their own behavior, which may actually be eliciting certain types of responses. At one extreme are physicians whose patients nearly always admire and extol them. At the other extreme are physicians whose patients almost uniformly express discontent and resentment. In both instances, the physicians should examine their customary attitudes and practices.

The other caveat pertains to the interpretation of the patient's transference reactions. Interpretation should be reserved for the psychiatrist or other trained psychotherapist. Nonpsychiatric physicians may utilize their understanding to maintain their perspective and to deal more discerningly with patients but should refrain from making interpretations.

Bearing the aforementioned concepts and guidelines in mind, one may describe some significant ramifications of the transference. One of these ramifications is *displacement;* that is, the patient expresses feelings that he or she has for the physician toward someone else, often another member of the health care team. For example, on entering the physician's office, a patient may remark, "The nurse was so busy that I didn't think she could pick me out from all the other patients in the waiting room." He may thereby indicate that he feels that the physician is not sufficiently interested in him as an individual. The physician could tactfully inquire further into the patient's reactions to both the nurse and himself and help allay his feeling of being neglected.

Another important complication of the transference is *splitting.* In this phenomenon, some of the patient's feelings are transferred to one member of the health care team while other feelings are shifted to one or more different members. For example, a patient berates one physician as harsh and insensitive and praises another as kind and understanding. Leaving aside for the moment the actual differences among physicians, the patient may be inappropriately expressing all her resentful, hostile feelings toward one and all her trusting, affectionate feelings toward the other. She may fear, unconsciously, that she might lose her relationship with important, helping persons if she were to express mixed feelings of anger and love toward both of them. The split enables her to maintain the idea that at least one of her important relationships will be preserved.

In such a situation, the physicians should review their attitudes and behavior to see if there is anything they might do to reduce the patient's feelings of frustration and anger. Beyond that, it may be helpful for them, and especially for the one who is the recipient of the anger, to realize that the splitting of the transference is an unconscious means the patient utilizes in attempting to cope with a frightening situation.

Countertransference

The counterpart of patients' transference to physicians is found in physicians' *countertransference* to patients. Physicians' attitudes, feelings, and behavior sometimes reflect patterns derived from earlier situations in their own lives. Without recognition, for example, physicians may have reactions to certain patients that are similar to their reactions to their own mothers, fathers, siblings, grandparents, or even their own children. Such reactions are extremely common. They pose difficulties if they interfere with the physician's objectivity and judgment or evoke inappropriate emotional responses.

Since many, if not most, of these reactions are unconscious, or outside awareness, they easily

elude recognition. There are, however, clues to countertransference reactions that can alert the attentive physician. Most obvious are such passing thoughts as "She acts just like my grandmother," or such remarks as "If you were my father, I would certainly advise you to have this operation." The passing thought that the patient is reminiscent of one's grandmother might be lightly dismissed as simply denoting a resemblance in physical appearance or some idiosyncratic mannerism, but it may signify that the physician has emotional reactions to the patient similar to those he had to his grandmother during his childhood. Likewise, the comment that one would give the same advice to one's father, may appear to imply only that the physician is sincere and wholehearted in her recommendation; but it may also mean, at another level, that she is unconsciously reacting to the patient as if he were her father. Only introspection and reflection can determine the answers to such questions.

Other aspects of the physician's emotional state, speech, and behavior also give indications of unconscious feelings and conflicts. The most helpful general sign is the physician's acting (or contemplating acting) in a manner that departs from his or her usual procedure. One example is the minimization of a symptom that would ordinarily arouse suspicion of a serious condition. Other illustrative behaviors are: forgetting an appointment, not returning a patient's repeated telephone calls, being oversolicitous, feeling inexplicably irritated, and omitting the "non repeat" instruction from a prescription for dangerous drugs.

Noncompliance

The morbidity and mortality from cancer are significantly increased by patients' not following recommendations for treatment. A substantial number of patients show noncompliant behavior in regard to surgery, radiotherapy, and chemotherapy. It has been estimated that the rate of noncompliance for all patients on all types of medications over a prolonged period is about 50%.[69] There are no statistics available specifically for antineoplastic agents. It should be noted, however, that physicians generally underestimate the degree of noncompliance of their patients.

The term *noncompliance* has unfortunate connotations. On one level, it denotes merely that the patient has not followed the recommendations or advice of the physician. At another level, however, it implies that the patient is like a child who has defied a parent. While some patients may want to rely totally on their physician's word, many others resent an authoritarian attitude and wish to maintain their autonomy in the doctor–patient relationship. They do not wish to be regarded as "obeying" or "disobeying" the "doctor's orders" but rather as participating actively in decisions that will determine their health and even their survival.

In current-day practice, therefore, it would be preferable to replace the expressions "compliance" and "noncompliance" with the term *working alliance,* representing an idea found useful in psychoanalytic treatment.[34] The change is more than a semantic one; it implies a difference in concept. With a working alliance, physicians examine those aspects of their own and patients' attitudes that are conducive or deleterious to building a productive therapeutic relationship. In place of the traditional authoritarian structure, it fosters an atmosphere of thoughtful and active cooperation.

Despite these objections to the term noncompliance, the author will continue to employ it because of its widespread use.

Literature on Noncompliance

Along with the increased recognition of and interest in noncompliance, the literature on this topic has burgeoned in the past dozen years.[37,68] The entity receiving the most consideration by researchers is hypertension. Cancer has received relatively little attention; furthermore, of those articles concerned with cancer, the majority pertain to detection and prevention programs,[35,72,74] rather than to treatment regimens.[5]

A large number of studies have attempted to correlate noncompliance with demographic variables, such as age, sex, race, education, and economic level. The results of such research reveal no consistent correlations with any of those variables.[37]

On the other hand, studies of the therapeutic regimen have revealed a number of significant findings, which have been well summarized by Blackwell.[9] Contrary to popular belief, the frequency of dosage is not related to compliance. Frequency refers to the number of times per day that a patient is to take medication; research does not bear out the common idea that reducing that number will increase the patient's adherence to the prescription.

However, the complexity of the regimen does bear a significant relationship with noncompliance. Complexity of the regimen is defined as the number of drugs prescribed. When three or more different medications are prescribed, "compliance falls off sharply."[9] The factor of complexity is of particular relevance to oncologists, since chemotherapy often entails the concurrent use of several antineoplastic drugs as well as the intermittent use of antibiotics, antiemetics, and other medications. It is essential to ensure comprehension on the part of the patient through repeated oral and written instructions. Assiduous follow-up by inquiry and objective tests, where available, is also necessary.

Psychological Factors

One should not end one's inquiry into noncompliance with a consideration of only the frequency of dosage and the complexity of the therapeutic regimen. Furthermore, one should refrain from attributing the patient's aversion to treatment solely to the burdens of the proposed modality. Although it is true that certain operations (e.g., mastectomy) produce evident disfigurement and that certain drugs (e.g., cisplatin) evoke severe side effects, it is also true that only some patients refuse a particular treatment while others accept it and persevere despite the sequelae.

One must look further, therefore, into other variables pertaining to compliance.[1,5,27,80] Approaching the problem from a psychological standpoint, one may divide the factors into two major categories: the factors within the patient and the factors external to the patient.

Those aspects within the patient include: character type; conscious and unconscious ideas and feelings about illness, treatment, and death; defense mechanisms; and transference phenomena. The external aspects include a large number of factors, most significantly the behavior of the patient's family and the responses of the physicians. The patient's decision to start or continue treatment represents the *final common pathway* of all the complex forces originating within and outside the individual.[33]

The Patient's Character Several types of character can be identified, but only a few of the familiar types are described here. For example, some people are very orderly, meticulous, and obstinate and meet a new situation with a large number of doubts and questions, often repeated in different forms (the obsessive-compulsive character). Other individuals ordinarily exaggerate symptoms and display a dramatic response to emotionally evocative events, such as the recommendation of an operation (the hysterical character). Some people tend to regard every trying situation, such as elaborate diagnostic procedures, with suspicion and, carried further, with the misconception that they are being badly used or experimented upon (the paranoid character).

Ideas about Illness, Treatment, and Death Most people cannot view having cancer objectively. They harbor a host of varied ideas, notions, preconceptions, and phantasies regarding their illness and treatment. For example, some believe that surgery is necessarily "mutilating" and, beneath this, are afraid of some kind of sexual injury. Others think that they are being "poisoned" by drugs or "burned" by irradiation, each of those ideas laden with anxieties of being harmed rather than helped by the therapeutic procedures. An extreme example was presented by a middle-aged woman who was psychotic and believed that if she submitted to breast surgery, her system would be poisoned by the polluted city air.

Some patients perceive themselves as soldiers waging battle against "the enemy," cancer. In contrast, others picture themselves as passive pawns, or victims, being plowed down by an inexorable fate. Some people regard illness and treatment as "punishment" for real or, much more frequently, imagined wrongful deeds or "sinful" wishes. They may resist treatment because they believe they should be punished through illness and death. Still other patients yearn for death because they believe they will be reunited with a dead loved one.[12]

Defense Mechanisms In facing the real danger of cancer and the various ideas and phantasies that it evokes, patients often respond with anxiety, depression, and anger. In attempting to control those distressing emotions, they employ a large number of unconscious mental processes, referred to as defense mechanisms. From the clinical standpoint of the oncologist, the most prevalent and significant of these mechanisms is *denial*, which is the means whereby the individual "avoids becoming aware of some painful aspect of reality" or of unpleasant feelings.[59] A patient, for example, may observe a lump in the breast for years, be aware of its enlarging, and yet not seek medical advice until it is a massive, fungating lesion. In reporting the history, she may state that she had observed the lump but thought that it was "due

to a bump," that it was "not serious," or that it "would go away by itself."

The force of denial prevails in some people regardless of intelligence and educational level and despite widespread campaigns about the warning signs of cancer. Underlying the denial of illness is anxiety of large proportions, which may also be denied. Such denial impedes efforts at the prevention, detection, and early treatment of cancer.

Even when a patient has consulted a physician, the denial may persist and present difficulties in compliance. Some individuals equate receiving treatment with being ill. Their thinking involves more than the obvious connection of treatment with side effects such as nausea, vomiting, and weakness; it means attaching the concept of illness to the treatment itself. For example, a patient with cancer of the ovary who was resistant to taking cisplatin and other drugs stated, "If I take the medication, that means I'm sick. If I don't take the medication, that means that I'm not sick."[33]

Transference Phenomena As noted previously, transference reactions to the attending physician are of a wide variety. Often they present no evident problem, particularly if the transference is of a dependent, trustful type. On the other hand, certain reactions may thwart the treatment and be life threatening.[13,48] The following history illustrates how a patient's strong, defiant transference led to noncompliance with the therapeutic regimen:

> The patient, a 21-year-old youth with acute lymphatic leukemia, had been placed in remission through the use of chemotherapeutic agents (vincristine, prednisone, L-asparaginase, and daunorubicin) and then had been started on maintenance chemotherapy (methotrexate, 6-mercaptopurine, vincristine, and prednisone). After several months of being free of symptoms, however, he decided on his own to discontinue the drugs. He subsequently developed fever of 40.6°C and other fulminant symptoms, which necessitated his readmission to the hospital, where he was found to be in relapse.[33]
>
> The patient complained that he could not discuss his treatment with his oncologist because that supposedly elicited what he called an "emphatic, unpleasant response." He revealed that not only had he discontinued the maintenance chemotherapy unilaterally but also had surreptitiously omitted doses of the antileukemia drugs previously, during his first admission to the hospital. He often disappeared from the floor without notifying the nurses, so that he was not present when the oncologist made his rounds. When he did see the oncologist, he was frequently contentious and provocative. In short, it was felt that he had a defiant attitude toward the oncologist and that he acted out his rebelliousness by taking decisions about chemotherapy into his own hands.
>
> Knowledge of the patient's earlier history is helpful in understanding his more recent attitudes and behavior. The patient described his father as the dominant figure in the family. A successful, hard-working dentist of southern Protestant background, he reportedly exerted pressure on the patient to follow rules of conventional behavior and to pursue studies aimed toward a career.
>
> The patient responded to his father in two main ways. On the one hand, he agreed verbally to all of his father's standards. On the other hand, he began to follow his own interests, particularly flying an airplane and collecting and using high-powered rifles. His academic career was erratic, as he attended three colleges in two years; and after he left college, his occupational record was unstable. Thus, while overtly compliant with his father's ideas, he pursued his own independent life, often acting in ways that were defiant and rebellious.
>
> His attitude toward his father was recapitulated in his reactions to the oncologist. He regarded the oncologist as authoritarian, as he had earlier in life felt toward his father. While outwardly complying in part with the oncologist's directions, he covertly defied his intentions in many ways, most blatantly by discontinuing the chemotherapy unilaterally.

Family Relationships In many situations, the relatives of patients are helpful and supportive. They rally round the patient, who in turn is willing to accept their help. In other instances, the family interactions may present obstacles to the patient's treatment.[50,85] For example, the 21-year-old patient described above, psychologically an adolescent, was still intensely engaged in the dependence–independence conflict with his parents (as well as with the oncologist) and became embroiled in family disputes about his treatment.

In some married couples, questions of autonomy and control are at issue and may interfere with treatment decisions. Husband and wife may argue about which doctor should treat the patient and wage a power struggle about whether the patient should undergo a given form of treatment.

The Responses of the Physician Patients' relationships with physicians can be a source of courage, hope, sustenance, and understanding. The more physicians are attuned to the patient's character, defense mechanisms, transference reactions, and family relationships, the less likely they are to encounter noncompliance. Concomitantly, if physicians express interest in their patients as people and attempt to tailor their method of dealing with patients according to individual needs, they increase the probability of eliciting cooperation.

Nevertheless, even when physicians are empathic, caring, and responsive, some patients re-

main noncompliant. Then it is advisable to focus on some of the features of noncompliance previously delineated—particularly, denial and defiant transference.

Some physicians attempt to deal with denial in patients by direct confrontation and frightening threats. For example, they might say to a resistant patient, "If you don't take these drugs, you will be dead in six months." Often such a message only increases the patient's level of anxiety and intensifies his need to deny. It may antagonize the patient and provoke him to leave the doctor and seek unproven nostrums such as laetrile. Physicians are more effective when they convey a message of hope and realistic optimism along with assurances of continued care. Furthermore, by inquiring into the reasons the patient is avoiding treatment, they may discover a specific fear and be able to allay it.

The reactions in a defiant transference relationship range from subtle to flagrant. Understanding the transference helps oncologists to maintain their objectivity and to devise methods of managing better with patients. Direct confrontation and argument are of little avail. Instead, one should respect patients' strivings for autonomy, independence, and control (in situations in which patients feel that so much is outside of their control). One can attempt to enlist patients' intellect and cooperation. One can allow them to make certain decisions within the total context of the treatment plan. One can be flexible when a change will not alter measurably the therapeutic effects of the regimen but will enhance patients' feelings of taking an active part in their treatment.

Some of the external causes of noncompliance arise from deficiencies in the system of health care. Sometimes a patient is faced with the conflicting opinions of various physicians, for example, when one recommends chemotherapy while another favors surgery or radiotherapy. Whenever possible, the physicians should settle such differences among themselves, so that they can present a unified treatment plan.

Role of the Oncologist in Psychological Care

The psychological role of the oncologist extends beyond fostering compliance. It comprises providing comprehensive care attuned to the emotional as well as the physical needs of the patient.

The physician's tasks can be divided into two main categories. The first category consists of explicit medical functions: the diagnosis and treatment of the patient's disease. The second category includes certain implicit functions: the understanding of the sick person's predicament and distress, imparting the feeling that the patient is not alone in his or her desperate plight, and relieving the patient's emotional distress. There are specific methods through which the oncologist can accomplish these aims.

Patient–Doctor Communication

Communicating skillfully is one of the most useful techniques that a physician can develop. Effective communication encompasses more than simply talking with the patient. It requires, actually, listening more than talking—in particular, listening in an attentive, empathic manner. It means not just understanding what the other person expresses directly and clearly but also discerning clues to topics that the person finds difficult to discuss. It often requires discreet inquiry into areas that one suspects are troubling but have not yet been revealed.

The conditions for this type of meaningful communication are worth noting. It is most important that doctors indicate by their entire manner that they are interested in listening. In the office, they should put away charts and papers and allow the patient to speak freely without untoward interruption. In the hospital, they should sit down at the bedside and convey the impression that they have all the time in the world, even if they can stay only five or ten minutes. In contrast, doctors who look restlessly at their watches or the door or speak of the many other patients they have to see "in the house" signify rapidly that they do not wish to be burdened by an extensive report of the patient's feelings.

Certain commonly used expressions set aside patient communication. For example, the frequently heard pronouncement "Everything is fine," uttered before the patient has had a chance to say anything, discourages the patient from expressing distress.

The use of technical language is usually a barrier to communication unless the patient has some scientific knowledge or the physician explains the meaning of the technical expressions. Terms such as "agammaglobulinemia," "plasmapheresis," and "terminal transferase negative" often confuse and

frighten patients and are better translated into phrases they can understand.

Even ordinary, nontechnical expressions need to be used cautiously because of the connotations they convey to some patients. For example, while the comment "You are not doing as well as we had expected" may signify to the physician that the patient's fever has risen or that the platelet count has decreased, to the patient it may carry a note of personal disapproval.

To many individuals, the expressions "doing well" and "not doing well" carry residues of meaning from the past—messages from parents, teachers, or others in authority who declared that the person was or was not living up to standards of behavior or performance. To some patients, the belief that they are meeting the expectations of the doctor—for example, in complying with an arduous chemotherapy protocol—may mean as much as "doing well" in regard to remission of their cancer. Hence, stating that a patient has "failed chemotherapy" has unfortunate emotional reverberations, often increasing the patient's guilt feelings.

Continuity and Coordination of Care

From a purely pragmatic standpoint, the advantage of a physician's caring for a patient throughout the course of the illness may appear self-evident. If one physician maintains the care of a patient, that physician has knowledge of the patient and the illness from the onset and is well informed about the treatments undertaken, the results obtained, and the complications encountered. Unfortunately, clinic and ward patients are frequently transferred from one doctor to another, increasing the potential for errors and delays in physical care. Concomitantly, the patients may suffer feelings of rejection and have problems in adapting to new physicians periodically. Such changes tend to increase the rate of noncompliance.

Even in private practice, the feeling of being cared for by one professional may be disturbed by contacts with the large number of physicians connected with the patient. It is common for an individual to have not only an oncologist but also a radiotherapist, a neurologist, an infectious disease expert, and several other subspecialists. Each specialist may communicate information of a different character or with a different emphasis that may confuse the patient and increase his or her anxiety. Hence, it is desirable that one physician be in charge and inform the patient about major developments concerning treatment, diagnostic tests, and general progress.

The unfortunate results of lack of coordination are illustrated by the following episode.

> The patient, a young married woman with carcinoma of the ovary, had undergone surgery and chemotherapy and then had a "second-look" exploratory laparotomy.[33] After the operation, she was told by the gynecologist that no trace of cancer had been found. Previously highly anxious and depressed, the patient now found renewed hope for her life. She made plans to resume her education and the care of her children. Two days later, however, another gynecologist informed her that the report of the peritoneal washings had just come back and revealed cancer cells. The patient responded with disbelief, followed by towering rage and recrudescence of her depression. It remained for her primary physician, a medical oncologist, and the psychiatrist who had been treating the patient, to attempt to restore her emotional equilibrium.

In contrast to the above example, establishing continuity and coordination of care provides significant advantages in regard to patients' emotional well-being. It helps to dispel one of the deepest fears of all individuals—the fear of abandonment. This fear, evident in childhood when there are separations from parents, is revived in the context of serious illness. Patients may fear that, as they become weaker and less able to manage by themselves, the important people in their lives will not stand by them. Those people include not only relatives and close friends but also the attending physician. Hence, if the physician can state explicitly that he or she intends to take care of the patient no matter what happens, the patient's feeling of security will be enhanced and anxiety will be lessened.

An important advance in the integration of patient care is the development of the *health care team* in some cancer centers.[10,50] The team consists of the various medical specialists, nurses, and representatives of the psychosocial disciplines (psychiatrists, psychologists, and social workers). Other caregivers with special skills, including physical and occupational therapists, nutritionists, teachers, and experts in vocational rehabilitation, may also contribute. Sometimes volunteers who are former patients or the relatives of former patients also participate.

With the team approach, all members share information, take part in the initial evaluation of the patient, and attempt to arrive at a consistent attitude to the patient and the family. Through regular meetings, they organize the various aspects

of the patient's ongoing care and avoid fragmentation.

They practice the "three C's" necessary for integrated, comprehensive care: communication, coordination, and continuity.

Knowledge of Patients' Characteristic Responses

Recognition of the patient's typical emotional responses to the physician, including transference reactions, helps in formulating an appropriate approach. For example, some patients have an unconscious need, predetermined by earlier experiences, to evoke guilt in the people who occupy important positions in their life. Other patients, especially those with masochistic tendencies, frequently attempt to provoke the doctor to anger, thereby confirming their preconceived idea that they are being badly treated. The physician's remaining calm and objective—and not responding unduly with signs of guilt, anger, or defensiveness—will often help to defuse such situations.

Perception of the patient's character traits also furnishes guidelines to management. For example, indecisive obsessional patients, who ask numerous questions, require detailed and repeated explanations about their condition and treatment; their level of anxiety is reduced by a primarily intellectual approach. On the other hand, hysterical patients who are dramatic and displaying signs of manifest anxiety may need a more global type of reassurance to the effect that their condition is well understood and everything feasible is being done. Paranoid patients need explicit explanations of procedures and the assurance that nothing will take place without their knowledge (that is, behind their backs).

A corollary of understanding the patient's reactions is the physician's recognition of his or her own mode of responding to particular types of patients. As mentioned previously, some of those reactions are based on countertransference. Others may be caused by more immediate circumstances, such as being overwhelmed by a large number of critically ill or dying patients.

Fostering Patients' Ability to Cope

Attention to the patient's defense mechanisms and coping mechanisms can help the patient mitigate the plight of having cancer. *Defense mechanisms* are unconscious mental means that serve to protect the patient against dangerous ideas, feelings, and impulses. *Coping mechanisms* are conscious, action-oriented, goal-directed methods of meeting the demands and challenges of reality. (The distinction between coping mechanisms and defense mechanisms, which appears to be valid to this author, is not made by most other writers on this subject; they either do not make this distinction or consider the defense mechanisms as one class of coping behavior.[10,20,47,85])

Only a few of the many, diverse defense mechanisms can be discussed in this presentation. *Denial* has been considered previously from the standpoint of its hindering compliance with therapeutic recommendations. On the other hand, denial sometimes serves an adaptive purpose, enabling patients to remain reasonably free of anxiety and depression and to continue with many of their usual activities while undergoing treatment.[8,71] In such cases, denial should be supported rather than challenged. As a general rule, denial in medical patients is not considered harmful unless it materially compromises the patients' acknowledgement of significant symptoms or impedes their taking the steps necessary for diagnosis and treatment.

Regression is a reversion to modes of functioning typical of an earlier period of one's life. It occurs very frequently, if not universally, in sick people, although the degree and form are highly variable. It is most evident in patients who have become dependent, clinging, and demanding, displaying transference reactions to physicians and nurses reminiscent of reactions they experienced as children toward their parents. The regression may help some individuals accept being ill and being realistically dependent on others. When the dependency goes beyond certain bounds, however, limits may have to be imposed. Furthermore, as patients recover from the acute phase of illness, they sometimes require encouragement to relinquish a regressive role and reestablish a more independent mode of operating. When they are no longer needed, special nurses may be discontinued. In hospitals that provide graduated care, patients often benefit from being transferred to a unit where they are responsible for more of their daily living activities. On returning home, they may need encouragement, reassurance, and praise to counteract the previous regression and return to a more mature level of behavior.

Displacement is another defense mechanism that is frequently encountered. It involves shifting

the emphasis from something that is basically significant and emotionally threatening to a relatively less significant, less threatening detail. For example, a patient with an advanced ovarian malignancy complained vehemently about the ecchymoses left by venipunctures while paying scant attention to the prodigious increase in her girth due to ascites. She thereby unconsciously avoided, or attempted to avoid, the anxiety about the progression of her cancer, which was endangering her life. Another common example is a patient's paying more attention to the appearance of the surgical scar than to the results of the operation—that is, whether all of the cancer was ostensibly removed. In such instances of displacement, the physician can support the defense mechanism by paying attention to the patient's professed concern, helping the patient divert anxiety from the more frightening issue. At the same time, of course, the physician should not be deterred from pursuing the primary physical problem.

Intellectualization and *rationalization* represent seemingly logical and thoughtful means of attempting to understand and explain threatening situations. They may serve the useful defensive purpose of diminishing anxiety. When, however, they are utilized to delay treatment or to seek out bizarre or quack remedies, they are maladaptive. They then need to be modified through identifying the underlying anxiety and employing an educational approach.

The defense mechanisms of intellectualization and rationalization should be distinguished from the coping mechanism of *obtaining information* through reliable sources. Such action may help patients deal more realistically and constructively with their illness and should be encouraged. A valuable source of informational materials is the annotated bibliography compiled by the Cancer Information Clearinghouse of the National Cancer Institute.[60]

In coping with disease, patients face the task of reconciling their level of aspiration with the limitations imposed by their physical and emotional condition. If the illness is disabling, patients must learn to adapt to a reduced level of competency and achievement. It is often helpful if physicians can outline what patients can reasonably expect of themselves—e.g., going to the office three hours a day instead of the usual eight hours. The *redefining of goals* in a realistic manner helps reduce the patients' feelings of failure and enhance their feelings of self-respect if they can meet the modified goals. It is also beneficial to some self-reliant individuals, who have difficulty in accepting the dependent role, to reassure them that under the circumstances it is a sign of strength to be able to alter one's usual pattern of independence and rely on other persons.

Additional means of coping include *finding acceptable substitutes* for actual or anticipated losses. For example, young patients treated with chemotherapy or castrative operations sometimes suffer anxiety and depression because of the threat of being deprived of parenthood. For men, the storage of sperm prior to treatment may ameliorate those feelings. Under appropriate circumstances, with patients of either sex, the possibility of adopting children may be broached. For women undergoing mastectomy, the potential of surgical reconstruction of the breast, whether or not actually realized, helps dispel feelings of loss. A significant aspect of all these procedures is that they engender, implicitly, hope for the future.

Helping Patients' Families

Those who are providing emotional support for the patient may themselves need encouragement and bolstering. Relatives who have been dependent on the patient may suddenly find themselves feeling helpless and anxious. They will require guidance and support from the physician, a social worker, or another member of the health care team.

When a patient is dying, some family members go through a process called *anticipatory grief*;[73] that is, they begin to mourn the loss of the loved one before it actually occurs. Sometimes this process results in a withdrawal of relatives from the patient during the terminal phase of the illness. Educating the family about the continuing needs of the patient is indicated; but if they remain removed, additional support by nurses and other personnel may have to be provided to the patient.

Dealing with Pain Problems

Pain problems are considered from two perspectives: the fear of pain and the treatment of pain syndromes. For many individuals, the fear of pain constitutes one of the greatest dreads connected with cancer. In some instances, for example, patients with leukemia and lymphoma, one can offer

reassurance that severe pain is relatively uncommon in their illnesses. With others, such as those with cancer of the prostate where pain due to bony metastases may be foreseen, one can reassure the patients that if pain develops later on they will be provided suitable relief and not be allowed to suffer needlessly.

In this connection, it is essential that the physician be dedicated to the viewpoint of furnishing adequate analgesia and have the knowledge commensurate with prescribing medications appropriately.[78] Despite numerous articles pointing out the misapprehension about addiction in patients with cancer, some physicians still do not prescribe narcotics in sufficient doses and at adequate intervals.[7,57]

It is well known that after prolonged use of morphine and similar drugs, patients do develop tolerance to the drugs and require steadily increasing amounts to obtain analgesia; but their need of drugs is different from that of street addicts craving a "high." Moralistic beliefs or misconceived notions about stoicism should not deter the physician from prescribing enough medication, with sufficient frequency, to relieve pain.

Morphine and its derivatives, however, can produce delirium, which is sometimes associated with disturbing symptoms, such as paranoid delusions and hallucinations. In some such cases, reduction of the dose of the narcotic and the concomitant use of haloperidol have resulted in clearing of the sensorium and reduction of the patient's emotional distress without compromise of pain relief.

Role of the Consultation–Liaison Psychiatrist

As the importance of comprehensive care has gained increased recognition, psychiatrists have been requested more frequently to participate in the care of patients with cancer. As even the most knowledgeable oncologists rely on consultants from other areas such as neurology and infectious diseases, they also call upon psychiatrists for their specialized information and skills. The functions of consultation–liaison psychiatrists can be grouped under four main headings: education, liaison, consultation, and treatment.

Education

By participating in oncology conferences, the psychiatrist can often inform the other clinicians about meaningful aspects of the patient's emotional status and family or social situation. From the history, for example, the psychiatrist may discern indications that the patient is significantly depressed or anxious. The psychiatrist may make inquiries about the support system available to the patient. Lectures that link psychiatric concepts to the practice of oncology are also valuable, but they should be supplemented with meetings where current problems regarding patients and their families are presented.

Liaison

In some hospitals, the psychiatrist becomes an integral member of the oncology service. Besides seeing some patients in formal consultation, psychiatrists see many others briefly on ward rounds or on clinic visits. In conferences with other physicians, social workers, and nurses, they can often formulate a particular approach or attitude that will help in the management of "difficult" patients. They strive for an atmosphere in which patients are treated as whole people, encompassing treatment of their psychological and social problems along with their physical illnesses.

Consultation

Although the emphasis in this chapter has been on methods the oncologist can utilize in treating patients with emotional problems, there are often situations in which the psychiatrist can provide a new dimension. Consultations may be indicated not only because of particularly distressing or persistent psychiatric symptoms but also because of certain kinds of problems in patient management.

For example, if a patient's depressive or anxiety symptoms or sensorial defects are unresponsive to the efforts of the nonpsychiatric staff, a psychiatrist should be called. Do not delay unnecessarily. Early intervention may prevent undue suffering. If a patient, for instance, reluctantly acknowledges that he cannot go on living or is actively contemplating suicide, one should not sweep aside his plaints with hasty reassurance but offer help through psychiatric consultation. If one waits until the patient has retreated to a state of abject hopelessness and helplessness, his plight may be insuperable.

Numerous requests for consultation are initiated because of problems in patient management. For example, some patients are markedly angry,

provocative, and contentious. Others have regressed excessively and make extraordinary demands on doctors, nurses, and aides. Many patients are noncompliant for a wide variety of reasons. Some seriously ill people express an unusually intense fear of dying and death.

Treatment

While sometimes the consultation is sufficient, complex or serious circumstances may require the services of the psychiatrist over a more prolonged period of time. People who face upheavals in their family lives, accentuated by the occurrence of cancer, need extensive assistance, consisting of individual psychotherapy and family sessions. Patients engaged in disruptive conflicts with their physicians frequently benefit from psychotherapy aimed at understanding the genesis of their transference reactions. Those persons experiencing fear of dying may desire psychotherapy during the terminal phase of their illnesses. Some individuals facing death wish to recount their life histories extensively to a professional who can listen with empathy and help them view their lives in perspective.[84]

In addition, patients with severe psychiatric conditions may require the sustained care of a psychiatrist. Besides psychotherapy, various drugs, such as the antidepressants and tranquilizers, may be employed when appropriate. Although those drugs can be administered by a nonpsychiatric physician, questions about indications, choice of medication, dosage, and toxic effects may warrant a specialist in this area.

Summary

In adopting the biopsychosocial model, physicians extend their knowledge of their patients' conditions at several levels of understanding. At the most comprehensive level, they view the patient as a person with ideas and feelings, affected by past and current experiences, rather than simply as the host of a disease. At a descriptive level, they recognize the patient's specific emotional and behavioral reactions to the different stages of illness, such as diagnosis, treatment phase, and relapse. While those reactions may sometimes be classified under the formal psychiatric diagnostic categories, such as depressive disorders, anxiety disorders, and organic brain syndromes, they also merit consideration as highly individualistic responses to disease and treatment. At a deeper level, physicians perceive the underlying psychodynamic aspects of the person, notably the individual's character, phantasies, defense mechanisms, and transference reactions.

The physician's integration of this knowledge into practice will augment his or her effectiveness in treating patients with cancer and helping them cope with the crises of illness.

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